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CONTENTS

The Correlation of the Reaction to Protein from Certain Mycobacteria (Paratubercle Bacilli) with the Reaction to Tuberculin (OT). JOSEPH D. ARONSON AND WILLIAM KRAUS..... 731

Studies on the Virulence of Tubercle Bacilli Using P^{32}-labeled Bacilli. I. The Comparison of Elimination *in Vivo* of P^{32} Derived from Heat-killed Virulent and Avirulent Tubercle Bacilli. YOSHIHIRE YAMAMURA, JUNKICHI TANI, TAKEO TERAI, AND YUICHI YAMAMURA.. 738

Tuberculosis in Medical Students at the University of Maryland. JAMES R. KARNS..... 746

The Incidence of Segmental Lesions in Primary Tuberculosis in Childhood. With Special Reference to the Effect of Chemotherapy. GERARD LORRIMAN AND FRANCIS J. BENTLEY..... 756

Lung Structure in Three Dimensions after Inflation and Fume Fixation. B. J. BLUMENTHAL AND H. G. BOREN..... 764

The Evolution and Early Results of Tracheal Fenestration. E. E. ROCKEY, S. A. THOMPSON, AND C. F. BLAZSIK..... 773

Pulmonary Resection for Drug-resistant Cavitary Tuberculosis Utilizing Ancillary Drugs. WATTS R. WEBB..... 780

Case Reports:

Enlargement of the Innominate and Subclavian Arteries Simulating Mediastinal Neoplasm. ROBERT A. GREEN..... 790

Toxic Psychosis due to Isoniazid. Report of Three Cases with Studies of Patterns of Isoniazid Inactivation in the Serum. AVRUM B. ORGANICK..... 799

Notes:

Evolution of Murine Leprosy. Y. T. CHANG..... 805

Differentiation of Human Tubercle Bacilli from Atypical Acid-fast Bacilli. III. Modification of the Niacin Test, Using Tween-Albumin Liquid Medium. K. KONNO AND A. J. SBARRA..... 810

The Effects of Mechanical Agitation on the Growth of the H37Ra Strain of *M. tuberculosis*. D. W. WEISS..... 813

A New Method of Counting the Viable Organisms in BCG Vaccine. B. B. DIENA *et al.*... 816

Correspondence: Chemotherapy in Pneumoconiosis Complicated by Tuberculosis. A. PINES AND J. H. R. RAMSAY..... 818

Books..... 819

American Trudeau Society:

Abstracts of Medical Papers Presented at the Annual Meeting, May 24–29, 1959..... 822

Notices..... 851

Index of Authors..... 853

Index of Subjects..... 856

Index of Abstracts..... 863

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THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

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THE CORRELATION OF THE REACTION TO PROTEIN FROM CERTAIN MYCOBACTERIA (PARATUBERCLE BACILLI) WITH THE REACTION TO TUBERCULIN (OT)[1]

JOSEPH D. ARONSON[2] AND WILLIAM KRAUS

(Received for publication January 28, 1959)

Introduction

Acid-fast bacteria, other than tubercle bacilli and *M. johnei*, have been found widely distributed in soil, water, moss, dairy products, oil, on grass and grain, and in musical instruments. Because the original description of many of these organisms is meager or incomplete, it is conceivable that some of the bacteria described by investigators as new species represent previously isolated mycobacteria.

There is increasing evidence that mycobacteria which differ from tubercle bacilli in their colonial, morphologic, and biochemical characteristics and in their pathogenicity for certain animals have been found in sputum, pulmonary lesions, lymph nodes, urine, blood, gastric washings, skin, and feces. The biologic characteristics of many of these mycobacteria have been compiled and described by Hauduroy (1) under the designation of "paratubercle bacilli."

The tuberculin reaction must be regarded only as a relatively specific reaction since there is increasing evidence that infection with a specific Mycobacterium does not necessarily induce specific hypersensitivity. Thus, there are no specific differences in the reaction to Old Tuberculin (OT) prepared from human and from bovine tubercle bacilli. Tuberculous chickens react not only to tuberculin from avian tubercle bacilli but also to tuberculin from mammalian cultures, while cattle suffering from Johne's disease show a greater degree of hypersensitivity to avian OT than to johnin.

B. and E. Lange (2) found that tuberculous guinea pigs reacted to the intracutaneous injection of turtle, frog, trumpet, timothy grass, and milk mycobacteria and to the tuberculin-like product from these cultures. S. Meyer (3), on the other hand, reported that tuberculin-negative children injected intragluteally with mycobacteria isolated from some cold-blooded animals failed to react to OT, but reacted to the tuberculin prepared from some of these mycobacteria. Long (4) and Long and Seyfarth (5) noted that the injection not only of OT but also of cultures of frog, grass, or smegma bacilli into the testes of tuberculous guinea pigs induced an acute inflammatory reaction with necrosis of spermatocytes and spermatids. Blumenberg (6) concluded that the histologic appearance of the local tuberculin reaction was nonspecific and could be elicited by the injection of *B. coli*. Boquet and Nègre (7) found that the Koch phenomenon could be elicited in tuberculous animals not only by mammalian and avian tubercle bacilli but also by some paratubercle bacilli. The tuberculin produced by a chromogenic culture which Cabasso and associates (8) isolated from a fly induced an intense local inflammatory reaction in tuberculous guinea pigs and proved fatal to the animals when injected intraperitoneally. McCarter, Getz, and Stiehm (9) found that a high percentage of persons who reacted to OT from human tubercle bacilli also reacted to purified protein derivative (PPD) from avian tubercle bacilli. They further concluded that avian PPD sensitized man, and that hypersensitivity to PPD prepared from either human or avian tubercle bacilli could be due to sensitization by other mycobacteria.

That the nonspecificity of the tuberculin re-

[1] From the Henry Phipps Institute, University of Pennsylvania, Philadelphia, Pennsylvania; and the Department of Health, Bureau of Tuberculosis Control, Harrisburg, Pennsylvania.

[2] Deceased.

action is not limited to mycobacteria is indicated by the observations of Zinnser and Tamiya (10) who noted that guinea pigs infected with *Br. abortus* reacted not only to abortin but also to OT. Aronson, Saylor, and Parr (11) found that a significant percentage of Indians living in southeastern Alaska, who showed a high level of hypersensitivity to PPD from human tubercle bacilli, also reacted to protein prepared from *Br. abortus*, despite the fact that the Indians did not use fresh milk and were not known to have been exposed to abortus-infected animals.

The more frequent use of cultural procedures for the isolation of tubercle bacilli from body fluids and from resected pulmonary lesions has resulted in the isolation of an increasing number of chromogenic and nonchromogenic mycobacteria which are nonpathogenic for guinea pigs but differ in morphologic, colonial, and biochemical characteristics from tubercle bacilli.

The present study was undertaken to determine whether or not proteins from mycobacteria isolated from pathologic lesions of man would induce specific, local, inflammatory reactions in tuberculous and nontuberculous persons, as well as to find the relationship of such reactions to the local reaction following the intracutaneous injection of OT.

Materials and Methods

Proteins from the following bacteria, which it is considered preferable to include under the term "paratubercle bacilli" rather than "yellow bacillus," "atypical mycobacteria," or "anonymous acid-fast organism," and which have been isolated from resected pulmonary lesions, lymph nodes, skin lesions, and sputum were used in this study.

Culture # 15: isolated from resected pulmonary lesion from *Case 1*, reported by Buhler and Pollak (12) as the "yellow bacillus." Culture is photochromogenic.

Culture # 106: isolated from mesenteric nodes and other organs from *Case 2*, reported by Buhler and Pollak (12) as the "yellow bacillus." Culture is photochromogenic.

Culture # 160: isolated from resected pulmonary lesion of a patient hospitalized at the Battey State Hospital, Rome, Georgia, "Battey type." Culture is chromogenic, especially on potato medium and on Sauton synthetic medium.

Culture # 222: isolated from resected pulmonary lesion of patient hospitalized at the Veterans Administration Hospital, Minneapolis, Minnesota. Culture is photochromogenic.

Culture # 440: isolated from resected pulmonary lesion from patient hospitalized at Trudeau Sanatorium, Saranac Lake, New York. Culture is photochromogenic.

Culture # Bl: Repeatedly isolated over a period of five years at Phipps Institute, University of Pennsylvania, from sputum of patient with bilateral apical cavities. Culture is photochromogenic.

Culture "Co": isolated from resected pulmonary lesion of patient hospitalized at Hospital of University of Pennsylvania, Philadelphia, Pennsylvania. Culture is scotochromogenic.

Culture M. balnei: isolated from tuberculoid skin lesions of persons who had used a swimming pool in Orebro, Sweden, and reported by Linell and Norden (13).

These cultures[3] were maintained on American Trudeau Society medium, free from any inhibiting dye, and on Sauton synthetic medium. Cultures were transplanted at approximately four-week intervals.

Preparation of Protein

The seed culture for the isolation of the protein was obtained by inoculating the surface of 50-ml. flasks containing 25 ml. of Sauton medium with a flake of a culture grown on American Trudeau Society medium or with the pellicle which had formed on 5 per cent glycerol-potato medium. The thin pellicle which developed and covered the surface of the flask was then transferred to the surface of flat 5-liter bottles containing 1,500 ml. of Sauton medium, pH 7.2. The cultures were maintained at 37.5° C. for six to eight weeks or longer until a heavy pellicle formed on the surface. By the end of that time the underlying culture medium had assumed a faint opalescent appearance except for the scotochromogen culture "Co" which was yellow. One or more flasks from each culture were steamed for two hours in an Arnold sterilizer while other flasks were not heated. The bacillary mass from both the unheated and heated cultures was removed by filtration through fine sterile silk, followed by filtration through a medium-grade sintered glass filter and finally through a Seitz filter.

Green's method (14) of isolating the protein was adopted, since he has reported that the protein content and potency of tuberculin protein, isolated by the addition of trichloroacetic acid directly to the culture filtrate, was equal to the method recommended by Seibert and Munday (15), which requires that the filtrate first be concentrated by ultrafiltration through collodion-impregnated alundum candles. Sufficient cold

[3] The cultures have been supplied by the following persons: Cultures # 15, 160, 222, and 440 by Dr. E. H. Runyon, Veterans Administration Hospital, Sunmount, New York; culture # 106 by Dr. Ann Pollak, Albert Einstein College of Medicine, Yeshiva University, New York, New York; and the *M. balnei* protein by Mr. A. B. Paterson, Veterinary Laboratory, Ministry of Agriculture, Weybridge, England.

40 per cent aqueous solution of trichloroacetic acid was added to the filtrate, which had been kept at 4° C., to give a final concentration of 4 per cent. The precipitate which formed was permitted to settle overnight in the cold, after which it was centrifuged, washed three times with cold 1 per cent trichloroacetic acid, followed by repeated washings with acetone and anhydrous ether. The precipitate was then dried and kept *in vacuo*. A concentrated stock solution containing 2.0 mg. per ml. of the protein was prepared by dissolving the powder in tenth normal sodium hydroxide and diluting with physiologic saline containing 0.25 per cent phenol. Sufficient tenth normal hydrochloric acid was added, when necessary, to give a final pH of 7.2. Finally, the protein solution was filtered through a fine sintered glass filter, tested for sterility, and stored at 4° C.

Fresh dilutions for testing were made from the concentrated protein as required. The protein from *M. balnei* which was received in powder form[3] was diluted as described above.

Observations

The required dilutions of each protein as well as the dilutions of OT[4] were prepared within twenty-four hours of use. Each protein was given a code letter and code color, and colored plastic tape was used to mark all glassware and syringes to prevent mixing of the syringes. The intracutaneous injections were made in the middle of the anterior surface of the arm, since Aronson and Taylor (16) found that reactions are more definite and fewer doubtful reactions result when injections are made in the denser skin of the arm rather than in the loose skin of the forearm.

The number of subjects receiving any one protein was selected at random and was roughly the ratio of the institution population to the number of proteins used. Each person received an intracutaneous injection with an arbitrary dose of 0.00002 mg. of the protein from one of the "paratubercle bacilli" simultaneously with an intracutaneous injection in the opposite arm of 0.01 mg. of OT. The injection sites were observed forty-eight hours later, the area of edema determined by measuring the two longest axes, and the thickness of edema was estimated. In those whose reactions were negative or equivocal to either the protein or OT, a second injection of either 0.0002 mg. of protein or 0.1 mg. of OT, or both, was administered and observed forty-eight hours later.

Patients in Tuberculosis Hospitals

This study was conducted on a group of 1,216 male and female patients in two Pennsylvania state tuberculosis sanatoriums, on 1,322 male inmates of a Pennsylvania state industrial school, and on 423 inmates of the same industrial school twelve weeks following BCG vaccination.

The results presented in table 1 indicate that the per cent distribution of those in the tuberculosis sanatoriums who reacted to 0.01 mg. of OT was relatively uniform for each group, ranging from 83.3 per cent to 96.2 per cent, an average of 91.6 per cent of reactions. Of those reactions to 0.01 mg. of OT, 1.9 per cent were recorded as 1-plus reactions; 72.8 per cent as 2-plus; 24.2 per cent as 3-plus; one, 4-plus; while 5 per cent were doubtful.

In contrast to the relatively uniform percentage of reactions to OT is the wide variation in the percentage who reacted to 0.00002 mg. of protein from the different "paratubercle bacilli." Thus, 14.5 per cent reacted to the protein from the heated culture "Bl" and 86.0 per cent were positive to the protein from the heated culture *M. balnei*. No person reacted to the protein from the unheated and heated culture #440 or to the protein from the unheated culture "Co," this despite the fact that a high percentage of those same persons reacted to 0.01 mg. of OT.

A significantly higher percentage of 1-plus reactions was observed following the injection of 0.00002 mg. of the different proteins than of 0.01 mg. of OT, and with but two exceptions a higher percentage of 2-plus reactions followed the use of protein than of OT. On the other hand, with the exception of the protein from *M. balnei*, a significantly lower percentage of the proteins than of OT gave 3-plus reactions, and no protein induced a 4-plus reaction. Slight or indefinite reactions were observed more frequently following the injection of the protein than of OT. The percentage of doubtful reactions varied from 8 per cent for the protein from *M. balnei* to 31 per cent with the protein from the heated culture "Bl."

The intensity of the local reaction to the different proteins was correlated with the intensity of the reaction to OT. In general, people with a higher hypersensitivity to OT showed more

[4] The Old Tuberculin used in this study was supplied by the Wyeth Company, Marietta, Pennsylvania, whose preparation has been found over a period of many years to be relatively uniform in potency.

TABLE 1

CORRELATION OF REACTIONS TO OT AND TO PROTEIN FROM SOME "PARATUBERCLE BACILLI" Tuberculosis Sanatoriums

Culture	Number Tested	Positive to 0.01 mg. OT		Positive to 0.1 mg. OT		Total Positive to OT		Positive to 0.00002 mg. of Protein		Positive to 0.0002 mg. of Protein		Total Positive to Protein	
		Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
15 U	91	81	89.0	6	60.0	87	96.5	64	70.3	20	74.0	84	93.2
106 U	99	94	94.5	4	80.0	98	99.0	63	63.6	31	86.0	94	94.9
106 H	93	84	90.5	9	100.0	93	100.0	43	46.2	45	91.8	88	94.6
160 U	90	83	92.2	4	57.0	87	96.7	37	41.1	31	58.5	68	75.5
160 H	87	80	92.1	4	57.0	84	96.6	64	73.9	19	82.6	83	95.4
222 U	86	81	94.2	3	60.0	84	97.7	72	83.9	9	64.2	81	94.2
222 H	90	85	94.4	5	100.0	90	100.0	47	52.2	38	88.4	85	94.4
440 U	80	78	97.5	1	50.0	79	98.7	0	—	0	—	0	—
440 H	71	68	95.8	3	100.0	71	100.0	0	—	12	17.0	12	16.4
Bl—U	105	88	84.0	14	82.4	102	97.1	48	45.7	48	84.1	96	91.4
Bl—H	97	81	83.3	12	75.0	93	95.9	14	14.5	52	63.2	66	65.0
Co—U	89	81	91.0	4	50.0	85	95.5	0	—	0	—	0	—
Co—H	92	76	92.7	4	66.6	80	97.6	47	57.2	20	59.0	67	81.7
M. balnei—H	50	48	96.2	1	50.0	49	98.0	43	86.0	5	71.5	48	96.0
Total	1,210	1,108	91.6	74	72.5	1,182	97.7						

U = Unheated culture; H = Heated culture.

marked reactions to the protein, although exceptions were noted.

The 102 persons who showed doubtful reactions or who failed to react to 0.01 mg. of OT were injected in the opposite arm with 0.1 mg. of OT. Of this group, 74 gave a definite reaction. Thus, of 1,210 patients in tuberculous sanatoriums, 1,182 (97.9 per cent) reacted to either 0.01 mg. or 0.1 mg. of OT.

It will be noted from table 1 that, of those who were re-tested with 0.0002 mg. of protein following doubtful or negative reactions to 0.00002 mg., generally an equal or greater percentage reacted to the larger dose than to the initial 0.00002 mg. No person injected with the larger dose of protein from the unheated cultures #440 and "Co" reacted, while only 17 per cent were positive to 0.0002 mg. of protein from heated #440.

The results of this phase of the study indicate that, in a high percentage of patients in two tuberculosis sanatoriums, proteins from most of the "paratubercle bacilli" capable of inducing local or general lesions in man can induce a definite local inflammatory reaction comparable with that induced by Old Tuberculin.

Subjects from the Industrial School

Comparative skin tests with OT and with proteins from different "paratubercle bacilli" were carried out on 1,322 young males, the total population of a Pennsylvania state industrial school (table 2). This group included newly admitted persons as well as inmates of longer residence, who had been tuberculin tested in the previous year or years and who had received BCG vaccine when negative. It is noted that 44.4 per cent of the 1,322 persons tested gave positive reactions to 0.01 mg. of OT. Persons who gave doubtful or negative reactions were reinjected with 0.1 mg. of OT, and 19.7 per cent of those gave positive reactions. The total per cent of reactions to both doses of tuberculin was 55.4.

As among the sanatorium patients, the percentage of reactions to the different proteins varied with the protein injected, but roughly paralleled the reactions to OT. In the industrial school one protein, that from *M. balnei*, gave a higher percentage of reactions than OT. Those proteins which had produced no reactions among the sanatorium patients gave from 1 to 7.6 per

TABLE 2

CORRELATION OF REACTIONS TO OT AND TO PROTEIN FROM SOME "PARATUBERCLE BACILLI" Male Industrial School

Culture	Number Tested	Positive to 0.01 mg. OT		Positive to 0.1 mg. OT		Total Positive to OT		Positive to 0.00002 mg. of Protein		Positive to 0.0002 mg. of Protein		Total Positive to Protein	
		Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
15 U	96	45	46.9	7	13.7	52	54.2	22	22.4	25	33.8	47	49.9
106 U	98	33	33.7	13	20.0	46	46.9	25	25.5	18	24.0	43	43.9
106 H	95	43	45.2	8	15.4	51	53.7	25	26.3	18	25.7	43	45.3
160 U	98	30	30.6	5	7.4	35	35.7	10	10.2	18	20.5	28	28.6
160 H	92	24	26.1	17	25.0	41	44.6	27	29.3	17	26.2	44	47.9
222 U	95	33	34.7	12	19.4	45	47.4	15	15.8	28	35.0	43	45.5
222 H	82	37	45.1	12	26.7	49	59.7	17	20.7	27	41.5	44	56.6
440 U	96	50	52.1	9	19.7	59	61.4	1	1.0	23	24.2	24	25.0
440 H	95	39	41.0	11	19.7	50	52.6	7	7.4	9	10.1	16	16.9
Bl—U	94	49	52.1	9	20.0	58	61.7	40	42.5	15	27.9	55	58.5
Bl—H	102	48	47.1	13	24.1	61	59.8	17	16.7	22	25.9	39	38.2
Co—U	79	38	48.1	7	17.1	45	57.0	6	7.6	NT	—		
Co—H	101	54	53.5	13	27.7	67	66.3	43	42.6	20	34.5	63	62.4
M. balnei—H	99	64	64.6	9	25.7	73	73.7	70	70.7	29	24.2	77	77.8
Total	1,322	587	44.4	145	19.7	732	55.4						

U = Unheated culture; H = Heated culture; NT = Not tested.

TABLE 3

CORRELATION OF REACTIONS TO OT AND TO PROTEIN FROM SOME "PARATUBERCLE BACILLI" FOLLOWING BCG VACCINATION

Culture	Number Tested	Positive to 0.1 mg. OT		Doubtful to 0.1 mg. OT		Positive to 0.00002 mg. of Protein		Doubtful to 0.00002 mg. of Protein		Positive to 0.0002 mg. of Protein		Doubtful to 0.0002 mg. of Protein	
		Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
15 U	36	32	88.9	4	11.1	6	16.7	11	30.6	17	47.2	9	25.0
106 U	51	48	94.1	3	5.9	10	19.6	5	9.8	16	31.8	8	15.7
106 H	41	37	90.2	4	9.8	8	19.5	6	14.6	15	36.6	4	9.8
160 U	39	39	100.0	0	—	6	15.4	8	20.5	6	15.4	12	31.8
160 H	79	69	87.3	9	11.4	20	25.3	6	7.6	40	50.6	10	12.7
222 U	15	15	100.0	0	—	5	33.1	2	13.3	9	60.0	0	—
222 H	24	22	91.6	1	4.2	1	4.2	6	25.0	10	41.7	3	12.5
440 U	31	30	96.8	1	3.2	2	6.5	0	—	0	—	0	—
440 H	35	33	94.3	1	2.9	0	—	6	17.1	2	5.7	2	5.7
Bl—U	34	30	88.2	4	11.2	12	35.3	9	32.1	7	20.6	6	17.6
Bl—H	38	36	94.7	2	5.3	1	2.6	4	16.5	3	9.7	6	19.4
Total	423	391	92.4	29	6.8								

U = Unheated; H = Heated.

Note: *M. balnei* protein was not used in this study but was used in a published study of Aronson, Taylor, and McGettigan (Am. Rev. Tubere., 1954, *70*, 71). *M. balnei* gave a high percentage of reactions following BCG vaccination.

cent positive reactions with 0.00002 mg. of the proteins.

When the results of the tests in the school were compiled by race, the Negro inmates showed a consistently higher percentage of reactions as well as more intense reactions than the white inmates, both to 0.01 mg. of OT and to 0.00002 mg. of the different proteins.

BCG-vaccinated Subjects

These 423 young males at the same industrial school were a group originally negative to 0.01 mg. of OT who were vaccinated with BCG (table 3). Twelve weeks later they were re-tested with OT and with all but three of the same proteins used previously in the institution. Only the 0.1 mg. dose of OT was used at the end of the twelve-week period after vaccination, but the initial dose of 0.00002 mg. of protein was used and was followed by 0.0002 mg. when the reaction was negative or doubtful. Doubtful reactions are tabulated for this group.

Here again the reaction to the protein varied with the culture used, and the variations were similar to those among the two other groups of persons. It was noted, however, that the total percentage of positive reactions to both doses of the protein was consistently less than the percentage of reactions to the 0.1 mg. dose of OT. Among the sanatorium patients and the 1,322 inmates of the industrial school previously tested, the total percentage of positive reactions to the different proteins was more nearly that of the total positive reactions to OT.

Summary

The results of this study indicate that the protein fractions from cultures of certain "paratubercle bacilli" isolated from pulmonary lesions show marked variations in their antigenicity, varying from zero per cent to 96.0 per cent positive reactions. The thermostability of the skin-reacting material varies with the different mycobacteria.

The results of simultaneous tests made with Old Tuberculin and protein from different "paratubercle bacilli" indicate some common antigenic relationship between typical tubercle bacilli and certain of the "paratubercle bacilli," including some chromogenic mycobacteria.

Sumario

La Correlación de la Reacción a la Proteína de Ciertas Micobacterias (Bacilos Paratuberculosos) con la Reacción a la Tuberculina (T.A)

Indican los resultados de este estudio que las fracciones proteínicas de los cultivos de ciertos "bacilos paratuberculosos" aislados de lesiones pulmonares muestran notables variaciones en su antigenicidad, oscilando de cero a 96.0 por ciento las reacciones positivas. La termoestabilidad del material cutirreactor varía con las distintas bacterias.

Los resultados de pruebas simultáneas verificadas con tuberculina antigua y con proteína procedente de distintos "bacilos paratuberculosos" indican alguna común relación antigénica entre típicos bacilos tuberculosos y ciertos "bacilos paratuberculosos," comprendiendo algunas micobacterias cromógenas.

Resume

Corrélation entre la réaction à la protéine de certaines mycobactéries (bacilles paratuberculeux) et la réaction à la tuberculine (de Koch)

Les résultats de cette étude indiquent que les fractions protéiques de cultures de certains "bacilles paratuberculeux", isolés de lésions pulmonaires, varient considérablement quant à leur antigénicité, les réactions positives varient de zéro pour cent à 96 pour cent. La thermostabilité du matériel donnant une réaction cutanée varie selon les différentes mycobactéries.

Les résultats de tests simultanés effectués avec la tuberculine de Koch et la protéine provenant de différents "bacilles paratuberculeux" indiquent une certaine relation antigénique commune entre les bacilles tuberculeux typiques et certains des "bacilles paratuberculeux", y compris certaines mycobactéries chromogènes.

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STUDIES ON THE VIRULENCE OF TUBERCLE BACILLI USING P^{32}-LABELED BACILLI[1]

I. The Comparison of Elimination *in Vivo* of P^{32} Derived from Heat-killed Virulent and Avirulent Tubercle Bacilli

YOSHIHIRE YAMAMURA, JUNKICHI TANI, TAKEO TERAI, AND YUICHI YAMAMURA

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Introduction

It is still a matter of controversy whether tuberculous lesions are caused by primary toxicity of tubercle bacilli or by the secondary toxicity based upon the allergic reaction. Many investigators have tried to ascertain the existence of primary toxic substances in tubercle bacilli, but the question is still not clearly solved.

New interest in this question has been aroused by the observations of Bloch and his associates. They obtained a toxic lipid material from petroleum-ether extract of virulent tubercle bacilli and BCG (1–3). The material termed "cord factor" had delayed toxicity for mice by the injection of relatively small doses, and inhibited the migration of leukocytes *in vitro*. The same material was also obtained from wax *C* of virulent tubercle bacilli and BCG (4, 5). This material was purified by chromatography, and the chemical structure of "cord factor" was determined as trehalose-6,6′-dimycolate by Noll and associates (6, 7). In another report, the characteristic effect of virulent tubercle bacilli on leukocytes was demonstrated by Allgöwer and Bloch (8). The migration of leukocytes *in vitro* was completely inhibited by heat-killed virulent bacilli, but was not affected by avirulent heat-killed organisms.

On the basis of these observations, it was supposed that the virulent tubercle bacilli, which have more toxic substances, are phagocytized, digested, and eliminated from the host more slowly than avirulent ones. In the present paper evidence is presented that reveals that the toxic lipid fraction of virulent bacilli plays an important role in resisting the destruction *in vivo* and impairing the elimination mechanism of the host. Tubercle bacilli labeled with P^{32} were used as tracers.

Material and Methods

Animal experiments: All experiments were performed with inbred male Swiss albino mice, strain Na-8, six weeks of age, weighing approximately 15 to 20 gm.

Bacterial strains: The following strains of mycobacteria were used: human tubercle bacilli, strains H37Rv (virulent) and H37Ra (avirulent); bovine tubercle bacilli, strain Miwa (virulent); attenuated strain BCG; avian strains Takeo and Jucho (both avirulent for mice); and *Mycobacterium* 607.[2]

Preparation of suspension of heat-killed tubercle bacilli labeled with P^{32} and defatted bacilli: The bacilli were grown on Sauton liquid medium containing 10 to 20 microcuries of $Na_2HP^{32}O_4$ per ml. When a thin veil of bacillary growth just covered the surface of the medium, the culture flask was placed in a water bath heated at 60°C. for one hour. The bacilli thus killed were harvested on filter paper and were washed thoroughly with distilled water until the filtrate had no radioactivity. The excess moisture was removed by pressing the bacterial mass between sheets of filter paper. The bacilli were weighed and ground in an agate mortar, and bacterial suspensions of 2 mg. (wet weight) per ml. of physiologic saline solution were prepared. Another portion of heat-killed bacilli was washed with distilled water, dried, and extracted with 100 volumes of an ethanol-ether (1:1) mixture for two days at 0°C. The bacillary mass was collected by centrifugation at 3,000 r.p.m. for fifteen minutes. The bacilli were re-extracted three times in the same manner. Bacterial suspensions of 0.5 mg. (dry weight) (0.25 mg. corresponding to 1.0 mg. wet weight) per ml. were prepared from the extracted cells. In the next step, the heat-killed bacilli extracted with ethanol-ether (1:1) were subsequently extracted with 100 volumes of chloroform at 0°C. for two days, and the chloroform-soluble fraction was removed through filter paper. The extraction was repeated three times, the bacilli were col-

[1] From the Research Laboratory of the National Sanatorium, Toneyama Hospital, Osaka, Japan.

[2] The strains H37Rv, H37Ra, BCG, and *M.* 607 were obtained from the Division of Tuberculosis, National Institute of Health, Tokyo. The strains Miwa, Takeo, and Jucho were obtained from Takeo Department of Tuberculosis, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan.

TABLE 1

DISTRIBUTION OF P^{32} IN MOUSE BODY AFTER THE INTRAVENOUS INJECTION OF 0.5 MG. (WET WEIGHT) OF HEAT-KILLED BACILLI LABELED WITH P^{32}

Strain	Radioactivity Injected	Number of Animal	Immediately after Injection	Twenty-Four Hours after Injection					
			Whole Body	Lungs	Liver	Spleen	Kidneys	Remainder	Total
	*c.p.m.**		*c.p.m.*	*c.p.m.*	*c.p.m.*	*c.p.m.*	*c.p.m.*	*c.p.m.*	*c.p.m.*
H37Rv		1	5,620						
		2	5,280						
	5,630	3		586	1,779	254	85	1,392	4,096
		4		434	1,835	299	83	1,002	3,653
		5		552	2,310	90	87	1,388	4,427
	Average		5,450	524	1,975	214	85	1,261	4,059
	Per cent of recovery		96.7	9.3	35.0	3.8	1.5	22.4	71.9

* Counts per minute

lected, and bacterial suspensions of 0.5 mg. (dry weight) per ml. were prepared.

Injection into mice: Twenty to 22 mice were injected intravenously with 0.25 ml. of bacterial suspensions which contained 0.5 mg. (wet weight) of the heat-killed P^{32}-labeled bacilli or 0.125 mg. (dry weight) of defatted bacilli.

Measurement of radioactivity in whole bodies and organs of mice: Four mice were sacrificed at intervals of about five days in accordance with the whole homogenate technique of Yamamura and associates as follows (9). After being killed with ether, 2 mice were chopped into several pieces with scissors and homogenized for a few minutes in an electric mixer[3] with 9 volumes of distilled water. The homogenate was filtered through folded gauze in order to remove the hair, teeth, and nails. Thirty milliliters of homogenate were poured in a beaker and dried under an infrared lamp. Two other mice were examined at autopsy, and their lungs, liver, kidneys, and spleen were homogenized separately. Ten milliliters of concentrated HCl and 4 ml. of concentrated HNO_3 were added to the dried homogenate which was heated in a boiling water-bath until the mixture of HCl and HNO_3 was evaporated. Then, 2 ml. of concentrated HNO_3 and 10 ml. of distilled water were added, heated again to the boiling point, and filtered. The residue was washed with 50 ml. of distilled water. The pH of this filtrate was adjusted to 7.0 with 30 per cent NH_4OH; then 25 ml. of ammonium molybdate reagent were added and heated at 60°C. for thirty minutes in a water bath. The resulting precipitate of ammonium phosphomolybdate was collected on a filter paper in an aluminum funnel (diameter 2.1 cm.), and the radioactivity of this precipitate was measured with a Geiger-Müller counter.

The radioactivity of the bacilli injected into each mouse was measured as follows: Twenty-five milliliters of bacterial suspension were taken in an aluminum dish (diameter 2.1 cm.) and dried under an infrared lamp. The radioactivity was counted by a Geiger-Müller counter. The appropriate corrections were applied for background, natural decay, and sample volume.

Thus, the rate of elimination of bacilli from mice bodies was followed by measuring the radioactivities in organs and whole bodies of mice.

RESULTS

Distribution of labeled bacilli in mouse body by intravenous injection: The P^{32}-labeled heat-killed bacterial suspensions were injected into the tail veins of mice. Immediately, or one day after the injection, mice were sacrificed and their radioactivity was measured. The results are shown in table 1. Almost all radioactivity injected was recovered from the whole body of mice immediately after the injection. One day after the injection, 35 per cent of the activity was located in the liver. The radioactivity in the lungs, kidneys, and spleen was too weak to obtain exact values. Therefore, in the following experiments, radioactivity was measured only in the whole homogenate and liver.

In vivo elimination of heat-killed P^{32}-labeled mycobacteria: Various strains of P^{32}-labeled myco-

[3] Kenmix Liquidizer, Model A 900.

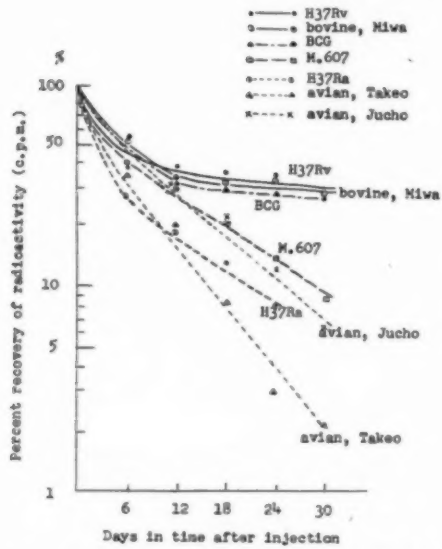

FIG. 1. Elimination of P^{32} from whole body of mice after the intravenous injection of 0.5 mg. of heat-killed P^{32}-labeled bacilli of various strains of mycobacteria.

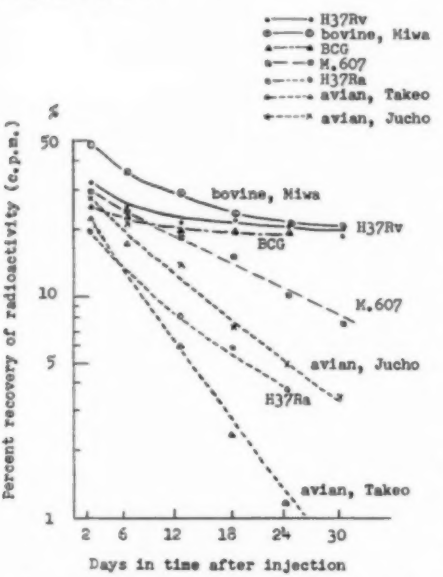

FIG. 2. Elimination of P^{32} from liver of mice after the intravenous injection of 0.5 mg. of heat-killed P^{32}-labeled bacilli of various strains of mycobacteria.

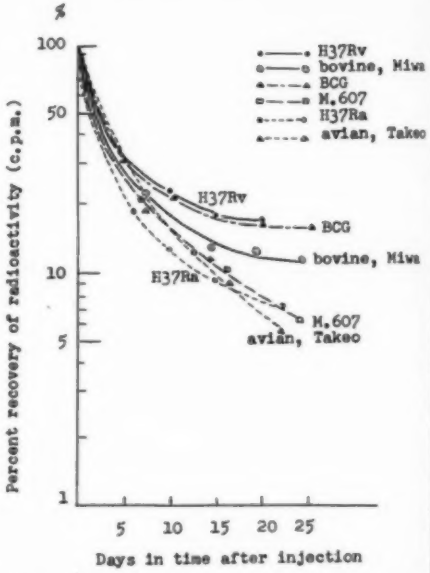

FIG. 3. Elimination of P^{32} from whole body of mice after the intravenous injection of 0.125 mg. (dry weight) of heat-killed P^{32}-labeled bacilli which were defatted by treatment with ether-alcohol (1:1) mixture.

bacteria were injected into the tail veins of mice and the rate of elimination of P^{32} was measured at constant periods of time after the injection.

The results are shown in figures 1 and 2. The elimination of P^{32}-labeled human tubercle bacilli, virulent strain H37Rv, the bovine virulent strain Miwa, and BCG from the whole body and liver of the mouse was slower than that of the human avirulent strain H37Ra and avian mycobacteria, the Takeo and Jucho strains. An intermediate rate of elimination was observed in the case of *M.* 607. It is notable that the results in the liver paralleled those in the whole body.

In vivo elimination of heat-killed P^{32}-labeled mycobacteria which were extracted with an alcohol-ether mixture: The results obtained when P^{32}-labeled mycobacteria which were extracted with alcohol-ether were used are indicated in figures 3 and 4. The differences in elimination from the whole body and the liver between H37Rv, Miwa strain, BCG, H37Ra, and the Takeo strain were significant, although they were less remarkable than with intact bacilli (*see* figures 1 and 2). Thus, the material responsible for the rate of P^{32} elimi-

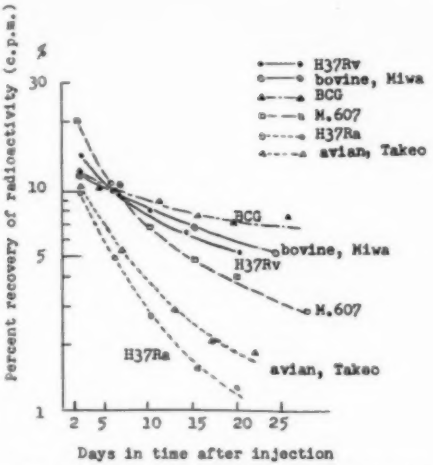

FIG. 4. Elimination of P^{32} from liver of mice after the intravenous injection of 0.125 mg. (dry weight) of heat-killed P^{32}-labeled bacilli which were defatted by treatment with ether-alcohol (1:1) mixture.

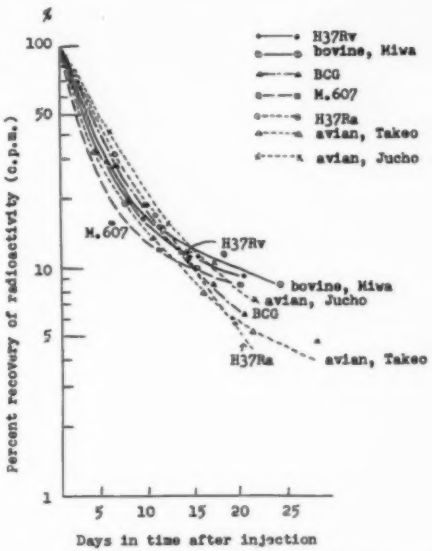

FIG. 5. Elimination of P^{32} from whole body of mice after the intravenous injection of 0.125 mg. (dry weight) of heat-killed P^{32}-labeled bacilli which were defatted by treatment with ether-alcohol (1:1) mixture and chloroform.

nation is not removed by extraction with alcohol-ether.

In vivo elimination of heat-killed P^{32}labeled mycobacteria which were extracted with alcohol-ether and chloroform: Various strains of heat-killed mycobacteria which were extracted with alcohol-ether and chloroform were injected into mice. The rate of elimination of P^{32} in the whole body and liver of the mouse is shown in figures 5 and 6. No differences were observed among 7 strains, irrespective of their virulence. These results indicate that extraction with chloroform has removed the material responsible for the difference in the rate of elimination.

The half-diminution times of P^{32} in various strains of heat-killed and defatted bacilli *in vivo* extracted with alcohol-ether and chloroform are shown in table 2. The half-diminution time was calculated on linear parts of the elimination curve of P^{32} at ten to thirty days after injection, and corrections were made for the natural decay of P^{32}. The data show that the half-diminution time *in vivo* of virulent bacilli labeled with P^{32} is three to five times longer than that of defatted bacilli and avirulent bacilli.

Control Experiments

In the course of these experiments, it was found that the P^{32}-labeled bacterial suspensions con-

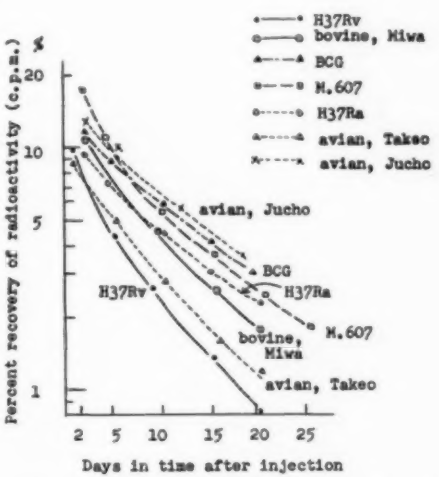

FIG. 6. Elimination of P^{32} from liver of mice after the intravenous injection of 0.125 mg. (dry weight) of heat-killed P^{32}-labeled bacilli which were defatted by treatment with ether-alcohol (1:1) mixture and chloroform.

TABLE 2

HALF-DIMINUTION TIME FROM WHOLE BODY AND LIVER AT 10 TO 30 DAYS AFTER THE INTRAVENOUS INJECTION OF HEAT-KILLED BACILLI OF VARIOUS STRAINS OF MYCOBACTERIA AND THOSE DEFATTED WITH ALCOHOL-ETHER (1:1) AND CHLOROFORM

(Natural decay of P^{32} is corrected)

Strain	Heat-killed Bacilli		With Alcohol-Ether (1:1) and Chloroform Defatted Heat-killed Bacilli	
	Whole Body	Liver	Whole Body	Liver
	days	*days*	*days*	*days*
H37Rv	47.0	57.0	11.5	5.5
Bovine, Miwa	43.0	25.5	12.0	7.5
BCG	31.5	54.0	10.0	10.0
H37Ra	10.5	10.5	7.0	11.5
M.607	9.5	16.5	15.5	10.0
Avian, Takeo	6.0	5.0	12.5	8.0
Avian, Jucho	8.5	9.0	9.0	8.5

TABLE 3

AMOUNTS OF NONBACILLARY WATER-SOLUBLE P^{32} CONTAINED IN HEAT-KILLED P^{32}-LABELED BACTERIAL SUSPENSIONS

Strain	Original Bacterial Suspension's Radioactivity (*A*)	Supernatant's Radioactivity of Bacterial Suspension (Nonbacillary Water-soluble P^{32}) (*B*)	(B)/(A)
	c.p.m./ml.*	*c.p.m./ml.*	
H37Rv	11,448	4,092	0.35
Takeo	9,595	2,178	0.23

* Counts per minute

tained some amount of nonbacillary water-soluble P^{32}. In order to estimate the effect of water-soluble P^{32} on the results, the following control experiment was performed.

The P^{32}-labeled bacterial suspensions, strains H37Rv and Takeo, were centrifuged at 35,000 r.p.m. for thirty minutes in a Spinco Model L centrifuge. The supernatant was stained by the Ziehl-Neelsen procedure and examined microscopically. No bacteria were seen. One-fourth milliliter of the supernatants and of the original bacterial suspensions, after being counted for radioactivity, was injected into the mice, and the rate of elimination of P^{32} from the mouse organism was observed.

As shown in table 3, the radioactivity of nonbacillary water-soluble P^{32} is about 20 to 35 per cent that of the original bacterial suspensions. The elimination of water-soluble P^{32} and of P^{32} from bacterial suspension from the mouse organism is illustrated in figures 7 and 8 (lines *A* and *B*). A true elimination line of the bacillary P^{32} can be calculated by substracting line *A* from line *B*. The resulting line is illustrated as line *C*

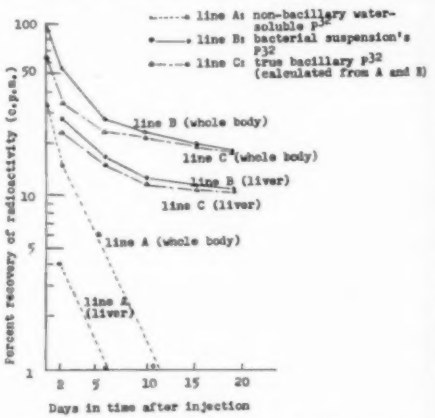
line A: non-bacillary water-soluble P32
line B: bacterial suspension's P32
line C: true bacillary P32 (calculated from A and B)
line B (whole body)
line C (whole body)
line B (liver)
line C (liver)
line A (whole body)
line A (liver)
Percent recovery of radioactivity (c.p.m.)
Days in time after injection

FIG. 7. Elimination rate of nonbacillary water-soluble P^{32}, bacterial suspension's P^{32}, and true bacillary P^{32} from bodies and livers of mice (in case of heat-killed H37Rv).

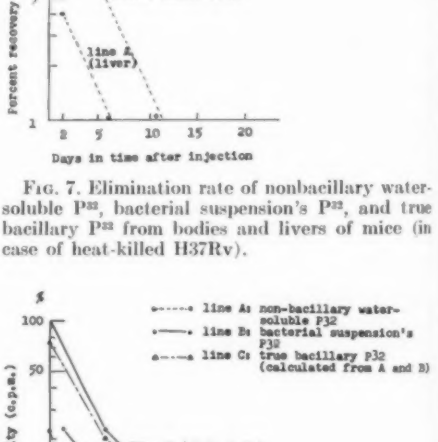
line A: non-bacillary water-soluble P32
line B: bacterial suspension's P32
line C: true bacillary P32 (calculated from A and B)
line B (whole body)
line C (whole body)
line B (liver)
line C (liver)
line A (whole body)
line A (liver)
Percent recovery of radioactivity (c.p.m.)
Days in time after injection

FIG. 8. Elimination rate of nonbacillary water-soluble P^{32}, bacterial suspensions P^{32}, and true bacillary P^{32} from bodies and livers of mice (in case of heat-killed avian mycobacteria Takeo).

in figures 7 and 8. A comparison of the lines *B* and *C* shows that the difference between the gradients of the two lines is negligible, so that the elimination rate can be compared with line *B* instead of with line *C*. In fact, the elimination of water-soluble P^{32} was so rapid that the results were not affected by the contamination with water-soluble P^{32}.

Discussion

It was reported by Bloch and associates that virulent tubercle bacilli and BCG had a toxic substance termed "cord factor" which was lacking in avirulent bacilli. This substance was obtained from the chloroform-soluble fraction of tubercle bacilli. Later, Spitznagel and co-workers (10) obtained a similar substance by extracting phenol-killed virulent tubercle bacilli with monochlorobenzene. Bloch and associates claim that this toxic substance soluble in monochlorobenzene is identical with the "cord factor" (11).

It was observed in the present investigation that the rate of elimination of heat-killed virulent bacilli and BCG was much slower than that of avirulent bacilli. This fact indicates that a toxic substance, which is responsible for the slow elimination, exists in virulent tubercle bacilli and BCG. While this substance was not removed by extraction with ether-alcohol, it was removed by chloroform extraction.

There have been some experiments using isotope-labeled bacilli. Ström (12) studied the Koch phenomenon by injecting P^{32}-labeled BCG, and observed that, when labeled BCG vaccine was injected intracutaneously into tuberculous patients or animals, the decrease of radioactivity in the injection papule was slower than was the case in healthy control patients or animals. Goldberg and Leif (13) employed aerosols of P^{32}-labeled organisms to determine the retention and initial distribution of *Pasteurella pestis* in mice. Kajiwara and co-workers (14) reported the distribution of the bacilli in bodies of mice after inhalation of aerosols, of different sizes, of P^{32}-labeled mycobacteria. Gerstl and associates (15) observed the breakdown *in vivo* of tubercle bacilli by host tissue with tubercle bacilli labeled with N^{15}. While these experiments were all carried out with living labeled bacilli, heat-killed labeled bacilli were used in the present work in order to eliminate the turnover of P^{32} due to metabolism and bacterial multiplication.

Summary

Tubercle bacilli labeled with P^{32} were obtained by culturing them in Sauton medium containing $Na_2P^{32}O_4$. Various strains of heat-killed P^{32}-labeled mycobacteria were injected intravenously into mice, and the breakdown of these bacilli *in vivo* was observed by tracing the radioactivity in the whole bodies and livers of mice.

In the case of the avian strains Takeo and Jucho (avirulent), and the human avirulent strain H37Ra, the rate of elimination of P^{32} from whole bodies and livers was fast; in the case of the virulent strains H37Rv and Miwa and the attenuated strain BCG, the rate of elimination was slow. The difference between H37Rv and Miwa and BCG was slight.

When the bacilli were extracted with ether-alcohol (1:1) according to Anderson's method and injected into mice, the results remained essentially unchanged, although the differences between virulent and avirulent strains were smaller than with unextracted killed bacilli.

When the bacilli were extracted with ether-alcohol followed by chloroform according to Anderson's method and then used for injection, the elimination rate of P^{32} from whole bodies and livers was almost the same for all strains of mycobacteria.

The calculated half-diminution time of P^{32} of virulent bacilli *in vivo* was three to five times longer than that of defatted and avirulent bacilli. This fact seems to show that virulent bacilli are more resistant to the *in vivo* destruction and elimination mechanism than the avirulent strain and that the chloroform-soluble lipid fraction plays an important role in this mechanism.

Sumario

Estudios de la Virulencia de los Bacilos Tuberculosos Usando Bacilos P^{32} Rotulados

Se obtuvieron bacilos tuberculosos rotulados P^{32} cultivándolos en medio de Sauton que contenía $Na_2P^{32}O_4$. Se inyectaron intravenosamente en ratones varias cepas de microbacterias matadas al calor y rotuladas P^{32}, observándose la desintegración *in vivo* de estos bacilos mediante el escrutinio de la radioactividad en los cuerpos íntegros y los hígados de los ratones.

En lo relativo a la cepa aviaria Takeo y Jucho (avirulenta) y a la cepa humana avirulenta H37Ra, la velocidad de la eliminación del P^{32} de los cuerpos íntegros y los hígados fué alta, y en lo relativo a la cepa virulenta H37Rv y la Miwa y la cepa atenuada BCG, la velocidad fué escasa. La diferencia entre La H37Rv y la Miwa y el BCG fué poca.

Cuando se extrajeron los bacilos con éter-alcohol (1:1) conforme a la técnica de Anderson y se inyectaron en ratones, los resultados permanecieron substancialmente inalterados, aunque las diferencias entre las cepas virulentas y avirulentas fueron menores que con los bacilos muertos y sin extraer.

Cuando se extrajeron los bacilos con éter-alcohol seguido de cloroformo, conforme a la técnica de Anderson, y se usaron entonces para inyección, el coeficiente de eliminación del P^{32} de los cuerpos íntegros y de los hígados fué casi idéntico para todas las cepas de micobacterias.

El hemitiempo de disminución calculado para los bacilos virulentos rotulados P^{32} fué *in vivo* de tres a cinco veces más largo que el de los bacilos desgrasados y los avirulentos. Este dato parece demostrar que los bacilos virulentos son más resistentes que la cepa avirulenta al mecanismo de destrucción *in vivo* y eliminación, y que la fracción lipida cloroformo-soluble desempeña un importante papel en este mecanismo.

Resume

Recherches sur la virulence des bacilles tuberculeux à l'aide de bacilles marqués avec P^{32}

Des bacilles tuberculeux marqués avec P^{32} ont été obtenus par culture dans le milieu Sauton renfermant $Na_2P^{32}O_4$. Diverses souches de mycobactéries tuées par la chaleur et marquées avec P^{32} ont été injectées par voie intraveineuse à des souris et la désagrégation *in vivo* de ces bacilles a été observée par traçage de la radioactivité dans le foie et dans tout l'organisme de l'animal.

Dans le cas de la souche aviaire, Takeo et Jucho (avirulente), et de la souche avirulente humaine H37Ra, la vitesse d'élimination de P^{32} du foie et du corps tout entier de l'animal était rapide; dans le cas de la souche virulente H37Rv et Miwa et de la souche atténuée BCG, la vitesse d'élimination était lente. La différence entre H37Rv et Miwa et BCG était minime.

Quand après extraction par l'éther-alcool (1:1), suivant la méthode d'Anderson, les bacilles étaient injectés à la souris, les résultats demeuraient essentiellement inchangés; toutefois, les différences entre les souches virulentes et avirulentes étaient moins marquées qu'avec les bacilles non extraits tués.

Quand les bacilles étaient extraits par l'éther-alcool, puis par le chloroforme, suivant la méthode d'Anderson, et utilisés ensuite pour l'injection, la vitesse d'élimination du P^{32} à partie du foie et du corps tout entier était sensiblement la même pour toutes les souches de mycobactéries.

La période calculée pour les bacilles virulents marqués par P^{32} *in vivo* était de 3 à 5 fois plus grande que celle des bacilles dégraissés et des bacilles avirulents. Ce fait semble démontrer que les bacilles virulents sont plus résistants au mécanisme de destruction et d'élimination *in vivo* que la souche avirulente et aussi que la fraction lipide soluble dans le chloroforme joue un rôle important dans ce mécanisme.

Acknowledgment

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TUBERCULOSIS IN MEDICAL STUDENTS AT THE UNIVERSITY OF MARYLAND[1]

JAMES R. KARNS

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Introduction

In September, 1934, a tuberculosis control program was started in the Student Health Service of the University of Maryland School of Medicine. The following report discusses the experience with the student body for the twenty-three-year period to June, 1957, in regard to trend of tuberculin sensitivity and attack rate of clinical tuberculosis while in medical school.

Material and Methods

More than 90 per cent of the students were tuberculin tested and all had chest roentgenograms on entrance to school. Re-testing was carried out on nonreactors at the beginning of each fall term. After 1948 students in the junior and senior classes were tested at both the beginning and the end of the school year, since these were years in which there was maximal exposure to patients. Those who were reactors on admission had chest roentgenograms annually, and converters had chest films every three months for a year from the time of conversion and at yearly intervals thereafter. Since 1948 all senior students have had chest roentgenograms at the end of the school year without regard to their tuberculin status.

Old Tuberculin (OT) was used for testing until October, 1944. From that time until the present, patch testing (Vollmer) has been used. The tuberculin tests were read by the physician in charge of the health service or by a nonmedical assistant trained by him. Only two physicians and two assistants have been involved during the period of the study. A reaction of 5 mm. or more of induration or 10 mm. or more of erythema to either 0.01 or 1.0 mg. of OT was interpreted as positive. Erythema, with or without vesiculation, was interpreted as a reaction to the patch test. Patch testing was shown to be a satisfactory method by a comparison study with one class which was also tested with first-strength purified protein derivative (PPD). Reporting on 609 cases of tuberculosis, Long (1) found that 94 per cent of the white and 96 per cent of the Negro patients reacted to either first-strength OT or PPD. Hence, converters should have sufficient allergy to tuberculoprotein to react to patch testing, 0.01 mg. of OT or of first-strength PPD. Use of the patch may have caused a few students with low degrees of allergy to be missed but has kept these subjects under the more strict surveillance accorded the nonreactors.

The number of students observed each year was corrected for students entering by transfer and for students who left school by means other than graduation. Only students who were observed for at least a full year were included in the study.

A program of BCG vaccination was considered in 1949 but was not instituted. A few students have been vaccinated with this material on their own initiative but not in sufficient numbers to affect the statistical results of this study.

The minimal criteria for a diagnosis of pulmonary tuberculosis were the finding of a characteristic roentgenogram and a tuberculin reaction. Usual clinical and laboratory procedures were performed on suspected cases, including at least three specimens of sputum or gastric washings with smear and culture for tubercle bacilli.

The year used to designate a class refers to the year in which that class entered school.

The accelerated wartime program began in June, 1942, involving the third and fourth years of the freshman class of 1940; the second, third, and fourth years of the class of 1941; and all subsequent years until its end in March, 1946.

Results

The percentage of reactors to tuberculin was tabulated for each class beginning with the academic year 1934–1935. The yearly percentage of reactors and the number of students tested each year are shown in table 1. This complex table is simplified in figure 1, which shows the percentage of reactors on matriculation and at the beginning of the fourth year for each class.

The yearly rate of conversion has been approximately the same except for the class entering in January, 1944, while the percentage of reactors on admission has diminished steadily over the period studied. The class which entered in January, 1944, had 39.3 per cent reactors on admission, 46 per cent at the beginning of the second year, and a sharp rise to 80.5 per cent at the beginning of the third year. Part of this increase was the result of 13 nonreactors' leaving school at the

[1] From the Student Health Service, University of Maryland School of Medicine, Baltimore, Maryland.

TABLE 1

PERCENTAGE OF TUBERCULIN REACTORS IN UNIVERSITY OF MARYLAND MEDICAL STUDENTS TESTED ANNUALLY, 1934–1957

Entrance Date	First Year		Second Year		Third Year		Fourth Year		End of Fourth Year	
	Number Tested	Per Cent of Reactors	Number Tested	Per Cent of Reactors	Number Tested	Per Cent of Reactors	Number Tested	Per Cent of Reactors	Number Tested	Per Cent of Reactors
September, 1934	107	65.4	99	73.7	97	79.3	95	81.1	94	84.0
September, 1935	89	55.0	77	66.2	85	71.7	83	77.1	78	84.6
September, 1936	90	57.7	85	63.5	90	65.5	89	77.5		
September, 1937	88	56.8	84	63.1	95	74.7	89	81.6	85	87.1
September, 1938	92	58.6	79	59.5	85	64.7	81	76.5	81	79.0
September, 1939	97	48.5	90	54.3	94	54.3	93	58.1	93	69.8
September, 1940	103	50.5	88	60.2	89	61.8	91	71.4	91	74.7
September, 1941	103	48.5	92	52.1	96	55.2	96	66.6	Not tested	
June, 1942	92	51.1	83	60.2	90	65.5	82	68.3	79	75.9
April, 1943	100	52.0	96	53.1	96	56.3			93	67.7
January, 1944	94	39.3	87	46.0	82	80.5	82	85.4		
October, 1944	94	26.6	85	41.2	80	41.2	75	49.3	75	50.6
September, 1945	82	37.8	68	39.7	71	46.4	68	52.9	68	57.3
September, 1946	88	34.1	77	48.1	78	52.6	72	58.3	72	65.2
September, 1947	100	38.0	92	41.3	96	47.9	96	53.1	96	56.2
September, 1948	100	26.0	90	30.0	95	43.2	95	49.4	95	52.6
September, 1949	101	36.6	93	41.9	95	58.9	95	60.0		
September, 1950	95	21.1	94	25.5	98	23.5	97	23.7	95	35.0
September, 1951	104	21.1	101	25.7	102	30.4	101	33.5	101	40.6
September, 1952	103	14.6	102	16.6	99	31.3	99	37.3	99	38.3
September, 1953	103	17.5	96	19.8	96	23.9	96	34.3		
September, 1954	88	30.6	84	34.5	79	36.7	76	42.1		
September, 1955	96	15.6	93	18.2	96	19.8				
September, 1956	93	11.8	96	15.6						
September, 1957	94	10.6								

end of the second year for scholastic reasons, but the major factor was that 22 students became tuberculin reactors. Four nonreactors and 5 reactors transferred from other medical schools to this class in its third year. Three crude plateaus are seen in the graph of positive reactors on entrance (figure 1), each plateau being lower than the preceding one. In the first plateau, the percentage of reactors among entering freshmen ranged between 65.4 and 48.5 per cent during the period September, 1934, to January, 1944. From January, 1944, to September, 1950, the percentage of reactors at entrance was 26 to 39.3 per cent. The percentage from September, 1950 to 1957 has been below 21 per cent except for the class entering in September, 1954, of which 30.6 per cent were reactors.

Experience with tuberculous infection as manifested by conversion of nonreactors is presented in table 2.

The number of nonreactors who converted each year gives the true incidence of infection for each

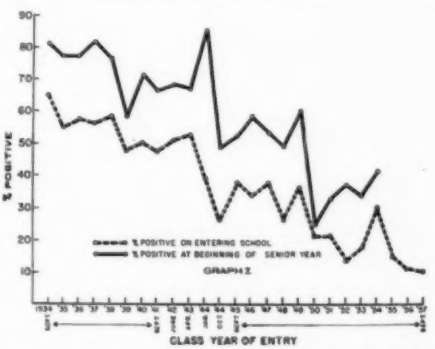

FIG. 1. Percentage of positive reactors at the beginning of freshman and senior years.

year in school. The nonreactors at matriculation who had converted when they returned to school for their second year were tabulated as the number of reactors at the end of the zero- to one-year period. The number of converters for the second and third years were tabulated at the

beginning of their third and fourth years of school. Conversion in each of the first three years was on the basis of a full year. The seniors who converted (shown by the three- to four-year period) did so over a nine-month period. During the wartime acceleration program (June, 1942, to March, 1946), all classes were tabulated for nine-month periods. When less than 90 per cent of any class was tested at graduation, it was not included in the three- to four-year period. This accounts for the lesser number of students for the fourth year. The classes were grouped as shown to provide larger groups for better statistical evaluation. Thus, in the period 1934–1939, zero to one year includes all freshman classes during those years.

There is little difference in "conversion" percentages for the groups until 1950–1954. The percentages in this group are definitely lower for each year in school than in any of the earlier groups, ranging from 3.7 per cent for freshmen to 7.4 per cent for seniors, and probably should be even lower as most of the positive reactors in the third and fourth years resulted from testing with second-strength PPD. In the first two groups (1934–1939 and 1940–1944), the third-year students show a higher rate of conversion. The 1945–1949 group shows a higher rate for the second year, but in the last group there is no significant difference in any of the years. The third and fourth years would have been expected to show a consistently higher rate, due to greater patient contact.

TABLE 2

TUBERCULOUS INFECTION EXPERIENCE OF MEDICAL STUDENTS, UNIVERSITY OF MARYLAND, 1934–1954

Period Following Admission	Number of Nonreactors at Beginning of Period	Number of Reactors at End of Period	Per Cent Converted
	1934–1939		
years			
0–1	216	29	13.4
1–2	177	17	9.6
2–3	172	34	19.8
3–4	136	11	8.0
	1940–1944		
0–1	289	35	12.1
1–2	233	38	16.3
2–3	211	45	21.3
3–4	92	8	8.7
	1945–1949		
0–1	275	23	8.3
1–2	244	38	15.6
2–3	216	27	12.4
3–4	189	14	7.4
	1950–1954		
0–1	375	14	3.7
1–2	361	25	6.9
2–3	335	24	7.1
3–4	202	15	7.4

Incidence of Clinical Tuberculosis

A survey of the health records of students from 1934 to 1957 revealed that 17 developed tuberculous disease while in school. The most striking feature of this survey was that 16 of these cases were diagnosed from 1934 to January, 1950, whereas only one case occurred after 1950. The student-years involved to June, 1950 were 5,521, giving a case rate of 2.89 per 1,000 per annum. In the seven years since 1950, only one case occurred, with 2,689 student-years involved. This gives a case rate of 0.37 per 1,000 per annum.

A summary of these cases, giving the tuberculin status on entry, the date of conversion, date of diagnosis of disease, and clinical features, is given in table 3.

Case 13 is of special interest because of the development of a tuberculous chancre two months after the student assisted at autopsies on tuberculous patients. A pulmonary lesion appeared four months after the cutaneous tuberculoma was removed. Diagnosis was proved histopathologically. The tuberculin reaction had been positive when the student entered school three years earlier and chest films were normal until four months after excision of the skin lesion.

Case 17 is of interest because it demonstrates the value of tuberculin conversion as an aid in diagnosis. The student was a nonreactor to tuberculin in his sophomore year. In the following summer he worked as a psychiatric aide in a Veterans Administration hospital. Chest roentgenograms taken at this hospital in June and September, 1953, were interpreted as normal. On returning to school his tuberculin reaction was positive and a chest film showed a minimal lesion in his left apex. The previous roentgenograms were obtained and the same lesion was found on the September film.

The attack rate according to tuberculin status at matriculation is shown in table 4*a* for the

TABLE 3

SUMMARY OF CASES OF ACTIVE TUBERCULOSIS

	Name and Sex	Class Year of Entrance	Entrance Tuberculin Test	Date of Conversion	Date Tuberculosis Diagnosed	Case Summary
1	B.I. (M)	1935	Positive		October, 1939	Chest films, November, 1935, February and December, 1938, showed increased hilar shadows with no change during this time. After graduation, was intern on TB service during summer. At end of this time roentgenogram showed bilateral apical infiltration, presumably tuberculosis. Sputum negative for tubercle bacilli, sedimentation rate normal. Entered sanatorium.
2	S.M. (M)	1936	Negative	October, 1939	February, 1940	Roentgenogram, February, 1940, showed increased hilar shadows and small infiltration in central portion of left lung. Roentgenogram of May, 1940, showed slight regression. Roentgenogram of September, 1940, showed bilateral infiltration with cavity. Infectious sputum. Sanatorium. Died one year later.
3	F.L. (M)	1937	Negative	June, 1940	June, 1940	Roentgenogram, June, 1940. Small infiltration right upper lobe. Sputum negative for tubercle bacilli. Subsequent chest films showed satisfactory arrest. Spent summer at rest.
4	A.A. (M)	1938	Positive	—	September, 1939	Roentgenogram normal, October, 1938. Chest film, September, 1939, showed infiltration extending from left hilum to periphery of lung at apex. Sputum positive for tubercle bacilli. Out of school one year. Treated by pneumothorax and phrenicolysis.
5	P.P. (M)	1938	Positive	—	October, 1941	Annual chest films negative 1938 to 1941. October, 1941, roentgenogram showed small infiltration in right upper lobe. Sputum examination and cultures negative for tubercle bacilli. Allowed to stay in school. Lesion arrested at time of graduation.
6	J.P. (M)	1940	Negative	May, 1943	November, 1943	Roentgenogram, 1940, normal. Roentgenogram, November, 1943, showed infiltration in the right upper lobe. Sedimentation rate 17. One sputum specimen positive for tubercle bacilli. Treated with bed rest at home. Graduated with class.
7	A.D. (M)	1942	Negative	December, 1944	January, 1945; Chest film of 1942, normal	Tuberculin negative March, 1944, positive in December, 1944. Roentgenogram of January, 1945 showed 10 cm. area of infiltration in the right upper lobe with cavitation. Treated in army hospital. Re-entered school in October, 1945, and graduated June, 1946.
8	J.B. (M)	1943	Negative	May, 1945	June, 1945; Chest film of June, 1943, normal	Apical lesion right upper lobe, pleurisy with effusion June, 1945. Hospitalized by army. Out of school one year. Re-entered in April, 1946, and graduated in 1947.
9	W.C. (M)	1944	Negative	December, 1945	January, 1946	Roentgenogram, January, 1946, infiltration left apex. No change on repeated films. Gastric washings negative for tubercle bacilli. Allowed to continue in school. Sedimentation rate normal.

(*continued*)

TABLE 3—CONTINUED

	Name and Sex	Class Year of Entrance	Entrance Tuberculin Test	Date of Conversion	Date Tuberculosis Diagnosed	Case Summary
10	J.L.R. (M)	1944	Positive	—	January, 1947	Small infiltration right upper lobe in January, 1947. Sedimentation rate normal. Serial roentgenograms showed no change. Hemoptysis September, 1948. Sanatorium treatment. Sputum and gastric washings negative for tubercle bacilli.
11	J.B. (M)	1944	Negative	October, 1948	October, 1948	Tuberculin negative March, 1948. Graduated in June, 1948. October, 1948 tuberculin positive and chest roentgenogram showed small area of infiltration in the left upper lobe. Subsequent films showed satisfactory arrest.
12	R.W. (M)	1945	Negative	November, 1947	February, 1949	Roentgenograms normal 1948 through December, 1948. February, 1949, early right apical lesion. One gastric washing positive for tubercle bacilli March, 1949, on culture and guinea pig. No fever. Sedimentation rates normal. Subsequent films showed improvement and resolution.
13	J.K.T. (M)	1945	Positive		1949	Roentgenograms normal until February, 1949. Assisted at autopsies on tuberculous patients in summer of 1948. November 27, 1948, tuberculous verruca excised from hand. Roentgenograms normal, January and February, 1949. April, 1949 infiltration in left upper lobe. Hospital and sanatorium care with arrest. Discharged September, 1949. Died in fire on Great Lakes steamer.
14	A.S. (M)	1945	Negative	August, 1948	August, 1948	Tuberculin negative November, 1947. Test not done in 1948. Developed acute exudative tuberculosis with cavitation during summer of internship, August, 1948. Thoracoplasty done with good result but complicated by homologous serum jaundice. Returned to school in 1950 and graduated in 1951.
15	G.K. (M)	1946	Negative	November, 1947	March, 1949	Roentgenograms normal until March, 1949. Two small areas of soft infiltration, right first interspace. Subsequent films showed regression and fibrosis. Sputum and gastric washings negative for tubercle bacilli. No time lost from school.
16	M.M. (F)	1946	Negative	November, 1949	January, 1950	Out of school 1947–1948 for personal reasons. Roentgenogram January, 1950, showed soft patchy infiltration with fibrosis, 2 cm. in diameter in the right upper lobe. Sputum and gastric washings negative for tubercle bacilli. Satisfactory arrest, by subsequent films. No time lost due to tuberculosis.
17	E.C. (M)	1950	Negative	November, 1953	November, 1953	Roentgenogram, October, 1950, was normal. Tuberculin test, negative in October, 1952. Worked in V.A. hospital from June to September, 1953. Roentgenogram was normal in June, 1953. Roentgenogram, September, 1953, showed small infiltration with calcification in the apex of the left upper lobe which was not seen until film was rechecked in November, 1953. Tuberculin test was positive in November, 1953. Sedimentation rate and gastric washings, negative. Treatment with Distrycin® and sodium PAS, restricted activity, and continued school.

group of students observed from 1934 to 1957. This does not include students who had clinically significant tuberculosis on entering school, being concerned only with the risk of the students' developing disease after entry. Five of 875 reactors developed tuberculosis, an attack rate of 5.71 per 1,000. The 1,427 nonreactors yielded 12 cases, an attack rate of 8.4 per 1,000. As 16 of the cases occurred from 1934 to 1950 and only one after 1950, the attack rate for the period of greatest incidence of disease is shown in table 4*b*. Five cases occurred in the group of 747 reactors, an attack rate of 6.69 per 1,000. Eleven cases occurred in the group of 873 nonreactors, an attack rate of 12.49 per 1,000. Since 1950, 128 reactors and 554 nonreactors at entry have been observed, the one case of tuberculosis occurring in the tuberculin-negative group.

In table 5 the time interval between tuberculin conversion and diagnosis is shown for the 12 cases which occurred in students who were tuberculin negative at entrance.

In 5 cases the first appearance of tuberculin sensitivity and roentgenographic evidence of disease were found simultaneously. Ten students presented evidence of disease within six months of demonstration of their becoming tuberculin reactors. No student had a negative patch test at the time tuberculosis was diagnosed. The severity of disease in relation to entrance tuberculin testing is shown in table 6.

In the group of students who were nonreactors on entrance to medical school, there developed: one case of pleurisy with a small apical infiltration, 10 cases of minimal pulmonary disease, and one case of moderately advanced pulmonary tuberculosis. Three cases of minimal and 2 of moderately advanced tuberculosis developed in the group of students who were reactors to tuberculin when they entered the school. Two of the latter group and 9 of the former group (nonreactors on admission) lost no time from school. One student who was a tuberculin reactor at matriculation lost six weeks at the end of his senior year, graduated with his class, and the disease became arrested after three months of sanatorium care. Two students who were reactors and 2 who were nonreactors on entrance lost a year of school and one nonreactor entrant lost two years. One of the students who developed minimal disease after having been a nonreactor at matriculation (*Case 2*) lost no time from school but, after two months of internship, had a bilateral apical infiltration with cavitation, entered a sanatorium, and died of tuberculosis a year later. This was the only death in the series.

TABLE 4*a*

ATTACK RATE ACCORDING TO TUBERCULIN STATUS OF CLASSES ENTERING, 1934 TO 1957

Status on Entry	Total Number of Students	Number of Cases	Attack Rate
Positive.....	875	5	5.71/1,000
Negative....	1,427	12	8.40/1,000

TABLE 4*b*

ATTACK RATE ACCORDING TO TUBERCULIN STATUS OF CLASSES ENTERING, 1934 TO 1950

Status on Entry	Total Number of Students	Number of Cases	Attack Rate
Positive.....	747	5	6.69/1,000
Negative....	873	11	12.49/1,000

TABLE 5

TIME INTERVAL IN MONTHS BETWEEN CONVERSION AND ROENTGENOGRAPHIC EVIDENCE OF TUBERCULOSIS

Months	0	1	2	4	6	15	16
Number of cases.	5	2	1	1	1	1	1

TABLE 6

SEVERITY OF DISEASE ACCORDING TO ENTRANCE TUBERCULIN

	Pleurisy	Minimal	Moderately Advanced
Negative.....	1	10	1
Positive.....		3	2

DISCUSSION

The percentage of students reacting to tuberculin on entering the University of Maryland Medical School has declined from 65.4 per cent in 1934 to 10.6 per cent in 1957. This decline has taken the form of three crude plateaus (*see* figure 1), the first from September, 1934, to January, 1944, with an average of 54 per cent of reactors; the second, from January, 1944, to

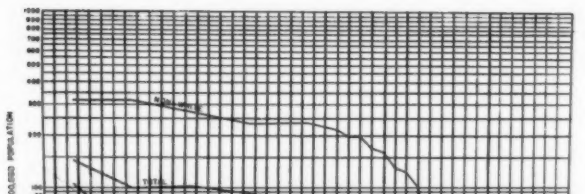

FIG. 2. Annual death rates from tuberculosis by race per 100,000 population, Baltimore City, Maryland, 1920–1956.

September, 1950, with an average of 34 per cent of reactors; and the third, from September, 1950, to September, 1957, with an average of 18 per cent of reactors. Reports from two other schools covering approximately the same years as the first plateau showed a much lower percentage of reactors. In the University of Buffalo Medical School from 1939 to 1946, Schuck and Aaron (2) found that an average of 23.4 per cent of the freshman classes reacted positively to first-strength OT or PPD. They attributed this low figure to use of the first-strength PPD and to the fact that many of their students came from small cities and rural areas. University of Wisconsin freshman students of classes graduating from 1940 through 1943 were reported by Dickie (3) as having an average of 27.4 per cent of reactors as freshmen in response to first- and second-strength OT. The higher average in the Maryland students is in keeping with the high mortality rate from tuberculosis in Baltimore during those years. The annual death rates from tuberculosis for Baltimore from 1922 to 1956 is shown in figure 2.[2] The decline of these rates is coincident with the decline in the number of reactors in the student population.

In the second plateau from January, 1944, to September, 1950, the average of tuberculin reactors fell to 34 per cent. All of the classes except the freshman class of January, 1944, were tested by the patch method. To eliminate the possibility of false-negatives occurring with patch testing, 53 students of one class who did not react to the patch test were tested with first-strength PPD, and only one reacted.

The further decline in the number of reactors in the freshman classes from September, 1950, to September, 1957, follows the trend in Navy and Marine recruits, aged seventeen to twenty, as reported by Drolet and Lowell (4). In the six years from 1949 to 1954, nearly one million young men, white and Negro, from all parts of the United States were tuberculin tested. From 1949 to 1952 reactors ranged between 9 and 11 per cent. In 1953, there were 5.3 per cent and in 1954, 4.6 per cent of reactors. The higher percentages in the medical students are presumably due to their average age, being four years older than the recruits, and to the fact that their pre-medical years afforded greater opportunity for contact with tuberculosis outside their home environment.

When average percentages of tuberculin reactors at the time of graduation (or at the beginning of the senior year if the class was not completely tested at graduation) are considered according to the previously mentioned plateaus, a similar result is seen. Reactors in the years of the first plateau increased by an average of 23 per cent to an average of 77 per cent at graduation. Those in the second plateau increased by an average of 27 per cent to an average of 61 per cent, and the third plateau by an average of 18 per cent to an average of 36 per cent of reactors at graduation. From this it appears that the rate

[2] Figure 2 (graph) by courtesy of Matthew L. Tayback, Director, Statistical Section, Baltimore City Health Department.

of conversion during four years of school has been the same since 1944.

Starting with the nonreactors in the freshman classes and tabulating the year-by-year conversion is a more accurate index of rate of conversion and shows a lower rate in the classes entering in the years 1950 to 1954, even though weighted by inclusion of several students who had reactions to second-strength PPD. No academic year has consistently shown a greater risk of infection than any other. Shuck and Aaron (2) made the same observation. A survey of tuberculin reactors in University of Chicago students registered in 1955 was reported by Szent-Györgyi (5). It was noted that medical students showed a higher percentage of conversion than did the whole student population.

For many years investigators have reported a high incidence of tuberculosis in medical students as compared with college students and other young adults. In 1953, Abruzzi and Hummel (6) reviewed the medical literature of the previous twenty years with attention to this problem and cited reports of incidence of tuberculosis among medical students ranging from 1.2 to 12.5 per cent, published from 1931 to 1948. By means of a survey letter they obtained information from sixty-two American schools on the incidence of tuberculosis, prevention, and control measures in those institutions for the period 1940 to 1950. This involved 166,959 student years and 557 cases of tuberculosis, giving a case rate of 3.34 per 1,000 per annum. Figures for the incidence of tuberculosis for a twenty- to twenty-nine-year age group of the general population were averaged for comparative purposes. This gave an average yearly case rate of 1 per 1,000 with a maximal yearly case rate conceivably to be expected (standard deviation on these figures $\pm$ 0.4) of 1.4 per 1,000. Using the same method of calculation, the University of Maryland students showed a case rate of 2.89 per 1,000 per annum up to June, 1950, which is comparable to the rate for medical students. Abruzzi and Hummel (6) report the very low or zero incidence of tuberculosis in a number of schools during recent years after institution of intensified control programs, some including the use of BCG vaccination. They strongly imply that BCG is largely responsible for this great reduction in incidence. However, they cite the experience of several schools which have enjoyed a low or zero incidence without BCG. Myers (7) presented a strong and comprehensive argument against BCG in 1952, and it was strengthened by his report with Boynton and Diehl (8) in 1957. The latter report states that in the past fourteen years only one medical student at the University of Minnesota developed a clinical lesion large enough to be detected by roentgenography. This and the occurrence of only one case at the University of Maryland in the past seven years points to factors other than BCG as being responsible for the reduced incidence in a school which had a high attack rate prior to 1950. Early diagnosis and treatment, improved methods of treatment, and management of tuberculosis as a contagious disease have brought about this reduction.

The higher incidence of tuberculous disease among the tuberculin converters has been similar to the experience of Dickie (3), Daniels and associates (9), and other early investigators. However, Shipman and Davis (10) found that most of their nurses who developed clinical tuberculosis during training were tuberculin reactors on entrance. Pollack and Cohen (11), and Israel and Long (12) found a similar rate of tuberculosis in reactors and nonreactors. A recent study by Palmer, Jablon, and Edwards (13) in Navy recruits showed that the chances of developing active tuberculosis today are five times greater for initially positive young men than for those who were nonreactors. These writers believe that these apparently opposite findings are not necessarily discrepant but result from a reduction in incidence of new infection. When tuberculin conversion rates were high, large numbers of nonreactors developed disease because morbidity rates are highest in the first months after conversion. The reactors had already passed through the period of highest morbidity and had probably lost the most susceptible members of the group. Thus, the number of new cases among the reactors, although they were at risk of endogenous as well as exogenous infection, seemed small by comparison. When the tuberculin conversion rates were low, endogenous reinfection rates in reactors seemed high because so few nonreactors were becoming infected.

No conclusions regarding severity of disease in relation to initial tuberculin status can be drawn from the present study because observation limited to the school years cannot present an accurate history of so chronic a disease as tuber-

culosis. The course of a case classed as minimal may change greatly after graduation removes the student from observation. Also, the number of cases is too small to develop any significant impressions. During the period of observation there was no significant difference in severity of disease or time lost from school in relation to entrance tuberculin. Badger and Ayvazian (14) followed a group of 745 nurses for five to fifteen years and observed 71 cases of significant tuberculosis. They found the same amount of disease appearing in those initially nonreactors and reactors and no difference in character, course, and prognosis.

Summary

A tuberculosis control program has been in operation at the University of Maryland School of Medicine since 1934. In this twenty-three-year period, 2,302 students have been adequately studied while in school.

During this period the number of students reacting to tuberculin at matriculation and graduation has diminished greatly, the lowest number of reactors occurring since 1950, indicating less risk of tuberculous infection in medical school than in previous years.

Seventeen cases of clinically significant tuberculosis have developed in students while in school, 16 of them before June, 1950, and only one from 1950 to 1957.

This recent low morbidity has occurred without the use of BCG vaccination. It is the result of early diagnosis and treatment, improved methods of treatment, and management of tuberculosis as a contagious disease.

The patch test was positive in all cases of clinically significant tuberculosis at the time of diagnosis.

In a group of 53 students the patch test was found to be as accurate as first-strength PPD.

Medical students still run a greater risk of tuberculous infection than other student populations, and continued vigilance is still necessary.

Sumario

La Tuberculosis en los Estudiantes de Medicine de la Universidad de Máryland

Un plan de lucha antituberculosa ha funcionado en Facultad de Medicina de la Universidad de Máryland desde 1934. En este período de veintitrés años, 2,302 estudiantes han sido estudiados adecuadamente mientras asistían a clases.

Durante este período, ha disminuído considerablemente el número de estudiantes que reaccionan a la tuberculina al matricularse y graduarse, observándose el número mínimo de reactores desde 1950, lo cual indica menos riesgo de infección tuberculosa en la escuela de medicina que en años anteriores.

Han aparecido 17 casos de tuberculosis clínicamente importante en los estudiantes mientras asistían a la escuela, 16 de ellos antes de junio de 1950, y solamente uno de 1950 a 1957.

Esta reciente morbidad baja ha tenido lugar sin el empleo de la vacunación con BCG. Ha sido efecto del diagnóstico y del tratamiento tempranos, de los mejores métodos de tratamiento y de la asistencia de la tuberculosis como enfermedad contagiosa.

La prueba del parche resultó positiva en todos los casos de tuberculosis clínicamente importante al hacerse el diagnóstico.

En un grupo de 53 estudiantes, la prueba del parche se mostró tan exacta como el DPP a su primera dilución.

Los estudiantes de medicina todavía corren un peligro mayor de infección tuberculosa que otros grupos de estudiantes y la vigilancia continua es todavía necesaria.

Resume

La tuberculose chez les étudiants en médecine à l'Université de Maryland

Une campagne antituberculeuse est en cours à l'Ecole de Médecine de l'Université de Maryland depuis 1934. Au cours de ces 23 ans, 2302 étudiants ont fait l'objet d'études adéquates pendant leur séjour à l'Ecole.

Durant cette période, le nombre des étudiants réagissant à la tuberculine au moment de l'immatriculation et de la sortie de l'Ecole a diminué considérablement, le nombre de sujets à réaction positive le plus bas a été obtenu à partir de 1950, indiquant un moindre risque de l'infection tuberculeuse à l'Ecole de Médecine qu'au cours des années précédentes.

Dix-sept cas de tuberculose avec signes cliniques significatifs se sont développés chez des étudiants pendant leur séjour à l'Ecole, 16 d'entre eux avant le mois de juin 1950 et le seulement entre 1950 et 1957.

Cette morbidité réduite récente s'est manifestée sans fair usage de la vaccination par le BCG; elle est le résultat d'un diagnostic et d'un traitement précoces, de méthodes de traitement améliorées et du traitement de la tuberculose comme une maladie contagieuse.

Le test du timbre tuberculinique était positif

dans tous les cas de tuberculose clinique apparente au moment du diagnostic.

Parmi un groupe de 53 étudiants le test du timbre a été trouvé aussi précis que le test au PPD à la première dilution.

Le risque d'infection tuberculeuse demeure toujours plus grand chez les étudiants en médecine que parmi les autres groupes d'étudiants et une vigilance continue est toujours nécessaire.

Acknowledgment

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THE INCIDENCE OF SEGMENTAL LESIONS IN PRIMARY TUBERCULOSIS IN CHILDHOOD[1]

With Special Reference to the Effect of Chemotherapy

GERARD LORRIMAN AND FRANCIS J. BENTLEY

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INTRODUCTION

While the necessity for giving chemotherapy in miliary tuberculosis and tuberculous meningitis is unquestionable, and a strong case can be made for its use in uncomplicated primary tuberculosis in childhood in order to prevent hematogenous spread, general experience has been that chemotherapy usually fails to modify the primary complex, judging roentgenographically. The British Medical Research Council (1) found an active primary complex with caseous pulmonary and lymph node lesions in 38 of 51 necropsies in children who died from tuberculous meningitis. These lesions showed no evidence of having been affected by streptomycin treatment that had been administered even over long periods. In some of these cases it is possible that the emergence of streptomycin-resistant tubercle bacilli was a factor, but the relative ineffectiveness of chemotherapy on the caseous component of a tuberculous lesion has now become well established. Lorber (2) was unable to see any roentgenographically demonstrable effects of streptomycin on the primary complex in 20 children with miliary tuberculosis and 2 children with tracheobronchial tuberculosis. Bentley, Grzybowski, and Benjamin (3) expressed disappointment in the immediate results of chemotherapy on the roentgenographic progress of primary pulmonary lesions, which appeared to resolve just as slowly as in the untreated cases; nor was the progressive contraction which commonly develops in segmental lesions prevented. Todd was unable to show any significant roentgenographic changes in primary complexes treated with para-aminosalicylcic acid (4) or with isoniazid (5); and Lincoln (6) considered that chemotherapy had no obvious effect on the resolution of primary tuberculosis. On the other hand, Miller (7) included among his indications for antimicrobial therapy a primary complex associated with acute febrile illness and rupture into a bronchus of tuberculous hilar lymph nodes. Moreover, Myers (8) claimed prompt relief from chemotherapy when bronchoscopy showed bronchial obstruction from pressure of enlarged hilar lymph nodes or when ulcerative lesions were seen in the bronchial tree.

Wallgren (9) showed that the mortality from tuberculosis fell from 35.9 per cent in infants less than one year of age to 4.4 per cent in those from three to seven years, and recommended (10) streptomycin for tuberculous infants during the first three months of their infection because this was the most dangerous period for dissemination. Debré (11) advocated streptomycin and para-aminosalicyclic acid (PAS) for all patients less than two years of age because of the difficulties in early detection of general dissemination and its bad prognosis. Hall (12) considered that streptomycin should be given to all patients less than one year of age or in cases of active endobronchial disease.

The general weight of evidence, therefore, seems to favor the view that antituberculous chemotherapy does not hasten the resolution of the primary complex as viewed roentgenographically, but that there is a strong case for giving chemotherapy to all infected patients less than two years of age. The case is likewise considered strong for giving chemotherapy to older children when recently infected (Bentley (13)) in order to prevent hematogenous spread. Implicit in this concept is the assumption that the primary complex, if not obviously benefited by chemotherapy, is at least not affected adversely to a significant degree. If, however, antimicrobial drugs were shown to have a detrimental action on the primary complex, this action and its consequences would have to be balanced against the known advantages of chemotherapy in uncomplicated cases in a particular age group before deciding whether or not to give antituberculous drugs.

In recent years the question has arisen whether there might not be adverse effects, particularly in the case of isoniazid. Plettenberg (14) re-

[1] From High Wood Hospital for Children, Brentwood, England.

ported 29 cases of lobar atelectasis among 182 children treated with isoniazid and one of the other antituberculous drugs, although he did not specify how many of the 182 had hilar lymph node enlargement. Four of the 29 died after bronchoscopy, at which caseous material was removed. Plettenberg suggested that treatment with isoniazid tends to precipitate rupture of enlarged tuberculous hilar lymph nodes into the bronchi, and that in treated cases lobar atelectasis takes longer to resolve than in those untreated. Choremis and associates (15) reported transient fever and increased pulmonary shadowing in a large proportion of primary complex cases about three weeks after treatment with isoniazid was started. Bentley (13) reported that, among 11 children less than two years of age recently admitted to High Wood with enlarged mediastinal lymph nodes only, segmental lesions developed in 5 of 9 who were given isoniazid and PAS, but in neither of the 2 untreated cases. On the other hand, Ryder (16) observed that only 4 segmental lesions developed among 50 Eskimo, Indian, and white children with uncomplicated mediastinal lymph node enlargement who were treated with antituberculous drugs. Ryder concluded that there is no obvious increase in the natural frequency of segmental lesions as the result of chemotherapy.

The present work was undertaken in order to study this problem further by comparing the incidence of segmental lesions which developed in children admitted to High Wood with the initial classification of simple primary pulmonary tuberculosis (enlarged hilar lymph nodes only or a simple primary complex) and treated with chemotherapy with those which occurred in a similar group not so treated.

Material

The investigation was confined to those children less than five years of age on admission who were discharged from High Wood between January, 1952, and July, 1957. The case notes and roentgenograms were studied, and in the great majority it has been possible (through the courtesy of the chest physicians concerned) to follow the subsequent clinical course and roentgenographic appearances up to the present.

For the period mentioned, 120 children who were cutaneous reactors to tuberculin and who were less than five years of age were recorded as showing on admission either enlarged hilar lymph nodes only or a primary complex, i.e., simple primary pulmonary tuberculosis. Thirteen of these (2 females less than two years of age and 5 males and 6 females between the ages of two and five years) were excluded from the investigation for the following reasons: In 8 cases there was either the certainty or strong suspicion of segmental lesions' having occurred at some time before admission. In 4 cases the hilar nodes were found to be already calcified on admission. In one case the history of tuberculosis went back as long as two years before admission.

Observations

Incidence of Segmental Lesions

The remaining 107 cases comprised 59 males and 48 females, among whom 26 patients (14 males and 12 females) developed segmental lesions—an over-all incidence of 24 per cent. The distribution by age and sex of the patients is shown in table 1. There was a greater incidence of segmental lesions in children less than two years old (34 per cent) than in the two- to five-year-old group (20 per cent), although the difference is not statistically significant.

Among the 107 patients, the hilar lymph node enlargement was on the right side in 50, on the left in 49, and bilateral in 8 patients. The distribution of segmental lesions is shown in table 2. There was a history of close contact with a case of active pulmonary tuberculosis in 83 of the 107 patients, i. e., 78 per cent. The contact was the mother of the patient in 37; the father in 32 (both parents in 2 cases); and other associates in 14 instances. Home conditions were recorded as bad in 31 cases. It was possible to determine the date of infection fairly accurately in 18 patients from either the occurrence of erythema nodosum or a history of the development of tuberculin hypersensitivity (Mantoux) between two known dates

TABLE 1

Total Incidence of Segmental Lesions in 107 Children Admitted with Simple Primary Pulmonary Tuberculosis

	0–2 Years		2–5 Years	
	Total Patients	Number Developing Segmental Lesions	Total Patients	Number Developing Segmental Lesions
Male	15	5	44	9
Female	17	6	31	6
Total	32	11	75	15

TABLE 2
ANATOMIC DISTRIBUTION OF SEGMENTAL LESIONS

Right Lung		Left Lung	
Upper lobe	8	Upper lobe	9
Middle lobe	10	Lower lobe	5
Lower lobe	4		

More than 1 lobe involved in 5 cases.

TABLE 3
COMPARABILITY OF TREATED AND UNTREATED GROUPS

	Treated		Untreated	
	Number	Per Cent	Number	Per Cent
Number of cases	41	100	50	100
Male	23	56	29	58
Female	18	44	21	42
Enlarged hilar lymph nodes only	25	61	30	60
Primary complex	16	39	20	40
Tuberculin sensitivity				
Positive to 0.1 TU	10	24	18	36
Positive to 1 TU	23	56	30	60
Positive to 10 TU	8	20	2	4
M. tuberculosis demonstrated	8	20	4	8
Gross hilar lymph node enlargement*	4	10	6	12
Close contact with active pulmonary tuberculosis	33	80	37	74
Bad home conditions	17	41	14	28
Mean age on admission	2.3 years		3.0 years	

* Gross lymph node enlargement: diameter from cardiac border greater than distance between upper border of seventh rib and lower border of eighth rib posteriorly

not more than four months apart. For these 18 patients the mean duration from infection to hospital admission was 17 weeks (range 8–40 weeks). Among them were 5 children who subsequently developed segmental lesions and for these the duration from infection to segmental lesion was respectively 32, 52, 19, 17, and 22 weeks. The mean was 28.4 weeks or, excluding the 52-week case (which is clearly an unusual occurrence) the mean was 22.5 weeks.

In comparing the incidence of segmental lesions among treated and untreated patients, a difficulty arises because 12 children were given one month's treatment with isoniazid alone as part of a clinical trial. Because of the brevity of such a course, it seems advisable to consider this group separately. Four other patients must be withdrawn for reasons which will be mentioned later. Ninety-one patients remain, 50 of whom were untreated; 41 were treated with antituberculous chemotherapy.

The comparability of the two groups is shown in table 3. In the treated group the mean age on admission was younger; the tuberculin sensitivity rather lower; tubercle bacilli (from sputum, laryngeal swab, gastric lavage, or bronchoscopic trap specimen) were recovered in a higher proportion of cases; and the incidence of bad home conditions was somewhat greater than in the untreated group. Nevertheless, the two groups are on the whole comparable except for the fact that the indications for the use of chemotherapy became progressively broader with the passage of time. Consequently, it may be seen in table 4 that, while 11 of the treated group were discharged between 1952 and 1954, and 30 between 1954 and 1957, the corresponding figures for the untreated group are respectively 38 and 12. In other words, there has been a greater tendency in recent than in former years to give chemotherapy to patients with uncomplicated primary disease.

The drugs used in the 41 patients comprising the treated group were isoniazid and PAS in 26; isoniazid and streptomycin in 4; isoniazid, PAS, and streptomycin in 2; streptomycin and PAS in 8; and PAS alone in one. In several cases *p*-aminosalicylic salt of isoniazid (Dipasic®) was also given, but only after an adequate course of one of the above-named regimens. This drug is no longer used at High Wood. The dosages employed for the various drugs depended on the child's weight and age, and the range for isoniazid was

TABLE 4
YEAR OF DISCHARGE FROM HIGH WOOD OF TREATED AND UNTREATED GROUPS

	Number of Treated Patients Discharged	Number of Untreated Patients Discharged
1952	6	10
1953	3	12
1954	2	16
1955	11	9
1956	7	3
1957 (to July)	12	—
Total	41	50

TABLE 5

INCIDENCE OF SEGMENTAL LESIONS IN 91 CHILDREN ADMITTED WITH SIMPLE PRIMARY PULMONARY TUBERCULOSIS

	Treated	Untreated
Number of patients	41	50
Number developing segmental lesions	10	10
Whole lobe affected	7	5
Less than a lobe affected	3	5
Mean time after admission segmental lesions developed	13.3 weeks	20.7 weeks
Range	7–30	8–40

50 to 150 mg., for PAS 3 to 8 gm., and for streptomycin, 0.25 to 1 gm. daily.

The incidence of segmental lesions in the two groups may be seen in table 5. Among both the treated and the untreated groups, 10 segmental lesions developed, those in the former group tending to occur earlier than in the latter; but the difference is not statistically significant. The date of infection could be estimated accurately in 3 cases. These were as follows:

A 2-year-old girl treated with isoniazid and PAS developed collapse–consolidation of the left upper lobe approximately nineteen weeks after tuberculous infection.

A 4-year-old boy, not given chemotherapy, developed a lesion in the apical segment of the left lower lobe and the apicoposterior segment of the left upper lobe approximately thirty-two weeks after tuberculous infection. A 2-year-old girl not given chemotherapy, developed collapse–consolidation of the whole left upper lobe one year after tuberculous infection. This was the longest interval between infection and segmental lesion in the whole series. The lesion was undoubtedly tuberculous, for tuberculous granulation tissue was aspirated from the obstructed bronchus at bronchoscopy.

In 8 of the 10 treated patients, the lesion developed while the patient was still receiving chemotherapy. In the remaining 2 patients, the lesion occurred respectively six weeks after stopping streptomycin–PAS, and seven weeks after stopping isoniazid–PAS.

The distribution of the segmental lesions by age and sex may be seen in table 6. There is clearly no difference in distribution between sexes, but a higher proportion of treated than untreated patients less than 2 years of age developed segmental lesions, while the reverse is true for the 2- to 5-year-old groups. These differences are not significant.

The 9 patients who, although given chemotherapy, did not receive isoniazid are included in table 6. As isoniazid is the drug primarily under suspicion of increasing the tendency to segmental lesions, the incidence of lesions in untreated cases is compared in table 7 with that of those receiving isoniazid along with either of the other two drugs. Seven of 16 children less than two years of age who received isoniazid developed segmental lesions compared with 2 of 10 untreated. This difference again is not statistically significant, nor is the difference for the 2- to 5-year-old group.

As previously noted, 12 patients were given one month's treatment with isoniazid alone. Two of these (both females) were less than two years of age, and one developed a segmental lesion. Of the remaining 10 patients between two and five

TABLE 6

AGE AND SEX DISTRIBUTION

	0–2 Years		2–5 Years	
	Untreated	Treated	Untreated	Treated
Male	5 (1)	9 (3)	24 (6)	14 (2)
Female	5 (1)	10 (4)	16 (2)	8 (1)
Total	10 (2)	19 (7)	40 (8)	22 (3)

Figures in brackets indicate those patients who developed segmental lesions.

TABLE 7

COMPARATIVE INCIDENCE OF SEGMENTAL LESIONS IN 50 UNTREATED PATIENTS AND IN 32 PATIENTS TREATED WITH ISONIAZID PLUS ANOTHER DRUG

	0–2 Years		2–5 Years	
	Untreated	Treated	Untreated	Treated
Male	5 (1)	7 (3)	24 (6)	9 (1)
Female	5 (1)	9 (4)	16 (2)	7 (1)
Total	10 (2)	16 (7)	40 (8)	16 (2)

Figures in brackets indicate those patients who developed segmental lesions.

years of age (6 male and 4 female), one male and one female child developed segmental lesions.

Whether these 12 patients were regarded as "treated," or classified as "untreated," and added to the appropriate columns in table 7, the difference in incidence of segmental lesions as between the children treated with isoniazid and those untreated would still not attain statistical significance. Mention was made earlier of 4 additional cases among the 107 which were excluded for purposes of comparison between the treated and untreated groups. They were the following:

A boy aged one year and nine months with gross paratracheal lymph node enlargement developed collapse–consolidation of the right upper lobe three days after beginning treatment with 100 mg. of isoniazid and 6 gm. of PAS daily.

A girl aged two years and four months developed erythema nodosum on March 5, 1953, and was found to have gross right hilar lymph node enlargement. She was given 0.75 gm. of streptomycin and 4 gm. of PAS daily at another hospital from March 10 to 24, and had no further treatment. Shortly afterward she was transferred to High Wood and on May 21, i.e., about seventeen weeks after the estimated date of infection, developed collapse–consolidation of the right middle and lower lobes.

A symptomless girl aged three years and four months was found on routine roentgenography, as a contact of her father, to have bilateral hilar lymph node enlargement, and there was also a suspicion of fine miliary shadowing. Five days after starting treatment with 0.5 gm. of streptomycin and 6 gm. of PAS daily, she developed collapse–consolidation of the anterior segment of the left upper lobe.

A girl aged four years and three months with lethargy and loss of weight was found to have left hilar lymph node enlargement. She was given the small dose of PAS, 3 gm. daily for three weeks, at another hospital, and was later transferred to High Wood, where she remained for five months more. No segmental lesion developed.

Although it may appear unlikely that treatment could be held responsible for the development of segmental lesions in the first 3 patients, none of the 4 can logically be assigned to either "treated" or "untreated" groups.

Total Duration of Observation

Among the 107 cases, the total duration of observation was less than six months in only one. For the 50 children in the untreated group the mean duration of observation was 3.4 years, as against 2.4 years for the 41 in the treated group; the difference between the means reflects the greater tendency to give chemotherapy in recent years. The total period of observation was less than one year for only 3 among the 50 untreated patients, and also for 3 among the 41 who received chemotherapy. As noted below, the mean interval between the hospital admission and development of segmental lesions for all cases combined was 15.5 weeks. Therefore, since the majority of patients were observed for more than a year, it seems unlikely that any significant number of late segmental lesions can have been missed.

Time Interval from Infection to Segmental Lesion

A few remaining points are of some interest. The mean interval between hospital admission and development of segmental lesions for all patients combined was 15.5 weeks (range one to 48 weeks). It has been mentioned previously that, for 18 patients in whom it was possible to estimate accurately the date of infection, the mean interval from infection to admission was 17 weeks (range 8 to 40 weeks). It is clearly not legitimate to add together these estimates for mean interval from infection to admission and admission to development of a segmental lesion, since only 5 patients belong to both groups. Nevertheless, the data do suggest that in the present series the mean interval from infection to development of a segmental lesion was probably much nearer 6 than 3 months.

Consecutive Segmental Lesions

In 3 cases a second segmental lesion followed the first after a considerable interval.

A girl aged twenty-three months on admission was found to have a symptomless right-sided primary complex on a routine roentgenogram obtained because her mother was tuberculous. The child was treated for twenty-one weeks with 4 gm. of PAS and 150 mg. of isoniazid daily, and was then discharged home. Seven weeks later, i.e., twenty-eight weeks after beginning treatment, she developed collapse–consolidation in the right middle lobe which cleared within six weeks. Subsequently, forty-seven weeks after the beginning of treatment, she developed a similar lesion in the anterior segment of the right upper lobe.

A boy aged two years and four months, examined as a symptomless contact of his mother, was found to have right hilar lymph node enlargement. He received no chemotherapy, and two months after admission (but five months after the hilar lymph node enlargement had first been noted) developed collapse–consolidation of the right

upper lobe and medial segment of the middle lobe. Resolution occurred in two months, but one year after the date of admission collapse–consolidation of the whole right middle lobe developed, and at bronchoscopy the lumen of the middle lobe bronchus was found to be occluded by granulation tissue which was removed. Re-aeration with considerable shrinkage quickly followed.

A 4-year-old girl, a symptomless contact of a tuberculous mother, was found to have a left primary complex. The child was given one month's treatment with isoniazid alone. Thirteen weeks after diagnosis and immediately after finishing isoniazid treatment for a month, collapse–consolidation of the apicoposterior segment of the left upper lobe developed, considerable re-aeration occurring within two months. Thirty-one weeks after the first abnormal roentgenogram, a similar lesion occurred in the anterior basic segment of the left lower lobe, and this re-aerated with some shrinkage within six weeks.

Other Findings

Symptoms at the onset of a segmental lesion were few. Fever of 100° to 102°F. for a day or two or lassitude occurred in only 2 of the 10 treated patients and in 5 of the 10 who received no treatment. Otherwise, the development of a segmental lesion was quite symptomless. Bronchoscopy was carried out at the time of development of the lesion in 5 treated and 4 untreated patients. In 2 of the treated patients, stenosis and edema were reported, and in the remaining 7 there was evidence of rupture of the lymph node into the bronchus. Among 13 patients (5 males and 8 females) in which gross lymph node enlargement was present, segmental lesions developed in 6.

Transient obstructive emphysema occurred in 3 treated patients, all less than eighteen months old, and 2 of these developed segmental lesions. One untreated girl, aged two years, developed obstructive emphysema of the whole left lung sixteen weeks after a left-sided primary focus was discovered. At bronchoscopy granulation tissue obstructing the left main bronchus was removed. The patient was observed for three years more, and no segmental lesion developed. Although this girl's case clearly could not be included among the segmental lesions, it is obviously only a matter of very slight difference in degree so far as the pathology was concerned.

Two untreated boys, aged respectively three and four years, developed pleural reactions after segmental lesions. In the first case the reaction was slight and developed seven weeks after the segmental lesion, while in the second a large pleural effusion accumulated fifteen months after a segmental lesion.

Discussion

The total incidence of segmental lesions (24 per cent) among the 107 cases of primary tuberculosis seems high, and comparison with other series is difficult. MacPherson and Lutwyche (17) found segmental lesions in only 39 (4.7 per cent) of 821 tuberculin-positive children attending a tuberculosis contact clinic. There was a direct correlation with age, however, for the incidence was 9.9 per cent for children less than four years of age, 5.9 per cent at four to eight years and 2.9 per cent at eight to twelve years. Clearly, not every child reacting to tuberculin would be found to have abnormal pulmonary shadows and, therefore, MacPherson and Lutwyche's figures of 9.9 per cent for children less than four years old may correlate reasonably well with that of 24 per cent in the present series. Thompson (18) reported 28 segmental lesions (4.3 per cent) among 655 children reacting to tuberculin, an almost identical figure to that of MacPherson and Lutwyche. There were 100 active primary pulmonary lesions among the 655; therefore, the segmental lesions accounted for 28 per cent of unselected active primary patients with visible lesions. Obviously, however, this figure is not comparable with the incidence in the present series.

Bentley and associates (3) found that, among 317 patients admitted with uncomplicated primary pulmonary tuberculosis, 19 developed segmental lesions, an incidence of 6 per cent. However, the mean age was just over 6 years compared with 2.7 years in the present series, and, as reported by MacPherson and Lutwyche, the incidence is much greater in young children. Moreover, the mean duration of illness before admission in that series of 317 cases was more than 3 months, whereas in the great majority of the present series the delay between diagnosis and admission was negligible. Since, in the present series, the mean interval from admission to development of the segmental lesion was 15.5 weeks, it is probable that a significant proportion of children in the series of Bentley and his associates developed segmental complications before admission. Taking these factors into account, there is again probably no great discrepancy between the figures of the Bentley series and those

of the present series. There was no difference with respect to sex in the incidence of segmental lesions in the present series, and the anatomic distribution corresponded with that of other series (MacPherson and Lutwyche (17), Bentley, Grzybowski, and Benjamin (3)).

In trying to assess the effect of chemotherapy on the incidence of segmental lesions, there are unfortunately too few cases to allow anything other than tentative conclusions to be deduced. A higher proportion of children less than two years of age treated with any combination of drugs, but especially if one drug was isoniazid, developed segmental lesions than did untreated children. This may represent a real trend, but in the present series it can be explained equally well on the basis of chance. Between the ages of two and five years it can be stated with some confidence that there is no evidence of any such adverse effect of chemotherapy; indeed the figures show rather the reverse. But again, the difference can be explained perfectly well by chance variations. With the steadily falling incidence of childhood tuberculosis in Great Britain, it will clearly be difficult to collect a sufficiently large series of comparable cases to obtain a decisive answer to the problem. On the other hand, it should be possible to collect a sufficiently large series of cases in other parts of the world, such as Asia. The organization of such an investigation would be by no means easy. In the light of present knowledge, there would seem to be little justification for withholding chemotherapy deliberately from children less than two years of age. Nevertheless there may be a sufficiently large number of well-documented cases in which drug treatment has not been administered even today to enable a clear answer to be obtained from a comparison with cases of patients now being treated.

Finally, the question must be faced of whether the possibility of chemotherapy provoking a greater incidence of segmental lesions in children less than two years of age is a sufficient reason for abandoning its use. Recently, an investigation carried out by the U. S. Public Health Service (19) into the prophylactic value of isoniazid in small children infected with tuberculosis has shown that 80 per cent of hematogenous complications can be prevented. Even if it were proved that chemotherapy resulted in a higher incidence of bronchiectatic segments or lobes, the high degree of protection it gives from such serious complications as miliary tuberculosis, tuberculous meningitis, renal and bone disease fully justifies its use in all recently infected children. In the case of children infected in the first year or two of life, chemotherapy should be considered imperative.

Summary

Among 107 consecutive unselected cases of simple primary pulmonary tuberculosis in patients less than five years of age on admission to High Wood Hospital for Children, who were discharged between January, 1952, and July, 1957, 26 (24 per cent) developed segmental lesions. For reasons discussed in the paper, 6 of these 26 children are excluded from the main analysis; another case occurred in a child given chemotherapy which did not include isoniazid. Considering only those children given isoniazid and another drug, 7 of 16 less than two years old (44 per cent) developed segmental lesions compared with 2 of 10 (20 per cent) given no chemotherapy.

For children between two and five years of age, the corresponding figures were two segmental lesions in 16 treated patients (12.5 per cent) and eight segmental lesions in 40 untreated patients (20 per cent). The numbers are too small for these differences to be statistically significant. It is believed that, even if chemotherapy produces a higher proportion of segmental lesions in children less than two years of age, such a disadvantage is outweighed by the advantage of preventing hematogenous spread of the disease.

Sumario

La Incidencia de Lesiones Segmentales en la Tuberculosis Primaria de la Infancia, con Referencia Particular al Efecto de la Quimioterapia

Entre 107 casos consecutivos y sin seleccionar de tuberculosis pulmonar primaria simple en enfermos de menos de cinco años de edad a su ingreso en el Hospital High Wood para Niños, que fueron dados de alta entre enero de 1952 y julio de 1957, 26 (24 por ciento) manifestaron lesiones segmentales. Por razones expuestas en este trabajo, se excluye del análisis a 6 de estos 26 niños; hubo otro caso en un niño que recibió quimioterapia, pero sin isoniacida. Considerando únicamente a los que recibieron isoniacida y otra droga, 7 de 16 niños de menos de dos años de edad (44 por ciento) manifestaron lesiones segmentales, comparado con 2 de 10 (20 por ciento) en los que no recibieron quimioterapia.

Para los niños de dos a cinco años de edad, las cifras correspondientes fueron dos lesiones segmentales en 16 enfermos tratados (12.5 por ciento) y ocho lesiones segmentales en 40 enfermos no tratados (20 por ciento). Los números son demasiado pequeños para conceder a estas diferencias importancia estadística. Parece que, aunque la quimioterapia produzca una proporción mayor de lesiones segmentales en los niños de menos de dos años de edad, esa desventaja queda contrapesada por la ventaja de impedir la difusión hematógena de la enfermedad.

Resume

L'incidence des lésions segmentaires dans la primo-infection tuberculeuse de l'enfance particulièrement à l'égard de l'effet de la chimiothérapie

Parmi 107 cas consécutifs, non sélectionnés, de tuberculose pulmonaire primitive chez des patients âgés de moins de cinq ans admis au High Wood Hospital for Children, et qui ont quitté l'hôpital entre janvier 1952 et juillet 1957, 26 (24%) ont présenté par la suite des lésions segmentaires. Pour les raisons discutées dans cet article, 6 de ces 26 enfants sont exclus de l'analyse principale; un autre cas de lésion segmentaire concernait un enfant soumis à une chimiothérapie ne comprenant pas l'isoniazide. En ne considérant que ceux des enfants recevant l'isoniazide et un autre médicament, 7 sur 16 d'entre eux âgés de moins de deux ans (44%) ont présenté des lésions segmentaires par comparaison avec 2 sur 10 (20%) parmi ceux non soumis à la chimiothérapie.

Pour les enfants de deux à cinq ans, les chiffres correspondants étaient de 2 lésions segmentaires sur 16 malades traités (12,5%), et 8 lésions segmentaires sur 40 patients non traités (20%). Ces chiffres sont trop faibles pour que ces différences aient une signification stastistique. Les auteurs estiment que même si la chimiothérapie entraîne une proportion plus élevée de lésions segmentaires chez les enfants âgés de moins de deux ans, l'avantage de prévenir la dissémination hématogène de la maladie l'emporte sur cet inconvénient.

Acknowledgment

The authors would like to thank Dr. W. J. Martin of the British Medical Research Council's Statistical Research Unit for kindly verifying their statistical tests of significance.

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LUNG STRUCTURE IN THREE DIMENSIONS AFTER INFLATION AND FUME FIXATION[1, 2]

B. J. BLUMENTHAL AND H. G. BOREN

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INTRODUCTION

The unique lability of the lung, as emphasized by Hartroft (1), does not allow it to be cut without gross distortion. To prevent such distortion from interfering with the study of terminal air spaces, rather elaborate techniques (2–8) have been devised but have not come into common usage. A method of formaldehyde fume fixation has been devised which provides a simple, practical means of studying the normal, as well as the abnormal, lung.

The method finds immediate usefulness in studying a diffuse disease such as emphysema, for it allows the evaluation of the types, distribution, and natural history of the morphologic changes in this disease. One lung may be examined by the method described while the opposite lung is studied by conventional methods.

The changes found in diverse types of pulmonary disease may be correlated with information gained by clinical, bacteriologic, or physiologic methods of study. Indeed, the morphologic findings as seen in three dimensions provide a common ground for all disciplines used in studying pulmonary disease. Moreover, the technique is an excellent screening method for determining whether pulmonary lesions can be experimentally produced.

METHODS AND MATERIALS

The basis of the method is to inflate the freshly obtained lung with a flow of air and to fix the lung with the vapor of formaldehyde present in the air. The equipment required consists of a source supplying 5 to 30 liters per minute of air, a ventilated hood, a flask or bottle of 1- to 4-liter capacity, 37 per cent formalin, rubber stoppers, rubber tubing, a ring stand, and clamps (figure 1). The rubber stopper (of a size slightly larger than the main stem bronchus) with a glass tube through it, is placed into the bronchus. The placement and subsequent securing of the stopper are facilitated by using three or four lengths of string which have been passed through the bronchial wall. An alternative method, which may also be used for single lobes of lungs, is the insertion of the wide end of a glass medicine dropper into the bronchus and securing it by a purse-string suture. Closure of larger vessels by a continuous suture prevents escape of blood as the lung is inflated.

The flask is partially filled with formalin, and a glass tube connecting to the air source is placed below the surface of the formalin. Air which has bubbled through the formalin leaves by a second opening in the top of the flask. Rubber tubing connects the outflow from the flask to the stopper in the bronchus. The flow of air is slowly begun and is increased until the lung expands to a position near maximal inspiration. The excess formaldehyde vapor leaves by small leaks on the lung surface.

The formaldehyde vapor is passed through the lung for three to five days. This is ample time for complete fixation. To prevent deflation and shrinkage of the lung, as well as to allow complete drying, air alone is then passed through the lung for an additional seven days. The passing of alcohol fumes through the lung results in a specimen more nearly normal in color.

After fixing, the lung is a rigid, dry, nonfragile specimen. It may be held by hand or by special devices so that sections 0.5 to 1.0 cm. in thickness may be obtained by cutting with a thin sharp blade. Paraffin impregnation is not required. These sections may be surveyed in their entirety by inspection with proper lighting. A convenient means of viewing these whole lung sections is to place them upon an X-ray view box. Areas which seem abnormal may then be examined by a dissecting microscope, without staining.

Selected sections of 0.3 to 0.5 mm. in thickness are cut by hand with a razor blade. Such sections may then be stained by classic staining techniques. To assure uniform contact of the staining liquids with all areas of the relatively thick specimen, small air bubbles are removed by applying a vacuum of 2,500 micro-atmospheres when the section is in contact with dyes, alcohol, or xylene.

[1] From the Department of Medicine, Baylor University College of Medicine, and the Veterans Administration Hospital, Houston, Texas.

[2] Presented before the Medical Session, as part of *Section 4B*, at the annual meeting of the American Trudeau Society, Chicago, Illinois, May 26, 1959.

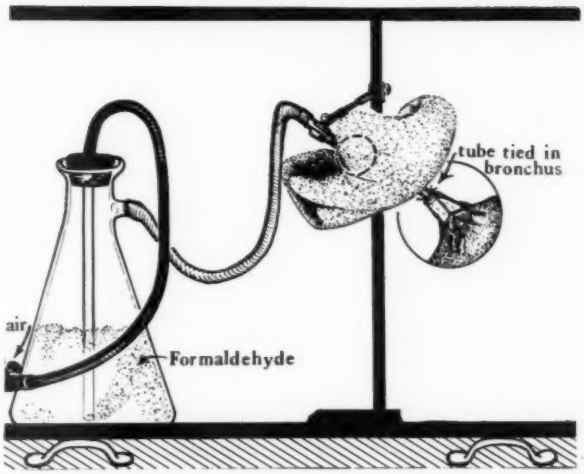

FIG. 1. A vapor of formaldehyde fumes obtained by bubbling air through formalin passes into and inflates the lung suspended by its bronchial attachment. The excess fumes are removed by a hood. The insert shows a method of attachment to the bronchus.

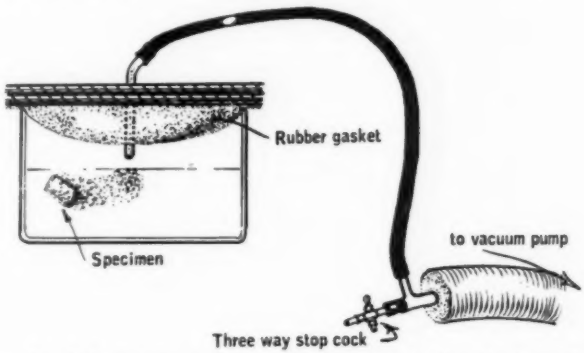

FIG. 2. Diagram of technique to evacuate a staining jar, illustrating the type of adaptor used. The adaptor consists of a ¾-inch plywood base to which a rubber gasket is glued. All jars are of the same size so that only one adaptor is required.

The manner by which vacuum is applied is illustrated in figure 2.

After the section has been cleared in xylene it is mounted in a commercially available plastic.[3] To remove xylene, the section is placed in uncatalyzed plastic and evacuated. In a separate container, a volume of plastic is mixed with hardener and evacuated. Glass slides 50 by 75 mm. in size are prepared by coating surfaces with a release agent.[4] One slide has a rim of applicator sticks to prevent compression of the specimen. Enough catalyzed evacuated plastic is poured over each slide to cover a surface with a thin layer. Both slides are placed in a 45°C. chamber for forty-five minutes to allow gelling. The lung section is placed in the container with the remainder of the catalyzed plastic and evacuated. In this manner catalyzed plastic fills the open spaces. The section is then placed upon the gelled base of the rimmed slide and covered by catalyzed plastic. The gelled surface of the unrimmed slide is placed over the surface, avoiding the trapping of air bubbles.

[3] Castolite®, the Castolite Company, Woodstock, Illinois.

[4] Vista Wax®, Simoniz Wax Company, Chicago, Illinois.

Gelling is repeated, and curing is accomplished by heating to 80° C. for one hour. Upon cooling, the glass slides will release from the plastic. Smooth surfaces are obtained which require no buffing and allow microscopic examination from either side. An alternate method of mounting is to cover the xylene-cleared specimen by balsam.

Results

Lung is obtained in a near-maximal inspiratory state which is rigid, dry, and nonfragile (figure 3, center). To provide evidence that formaldehyde fumes as obtained by this method are effective in fixing tissue, a study suggested by Dr. William Hentel was done. A large section

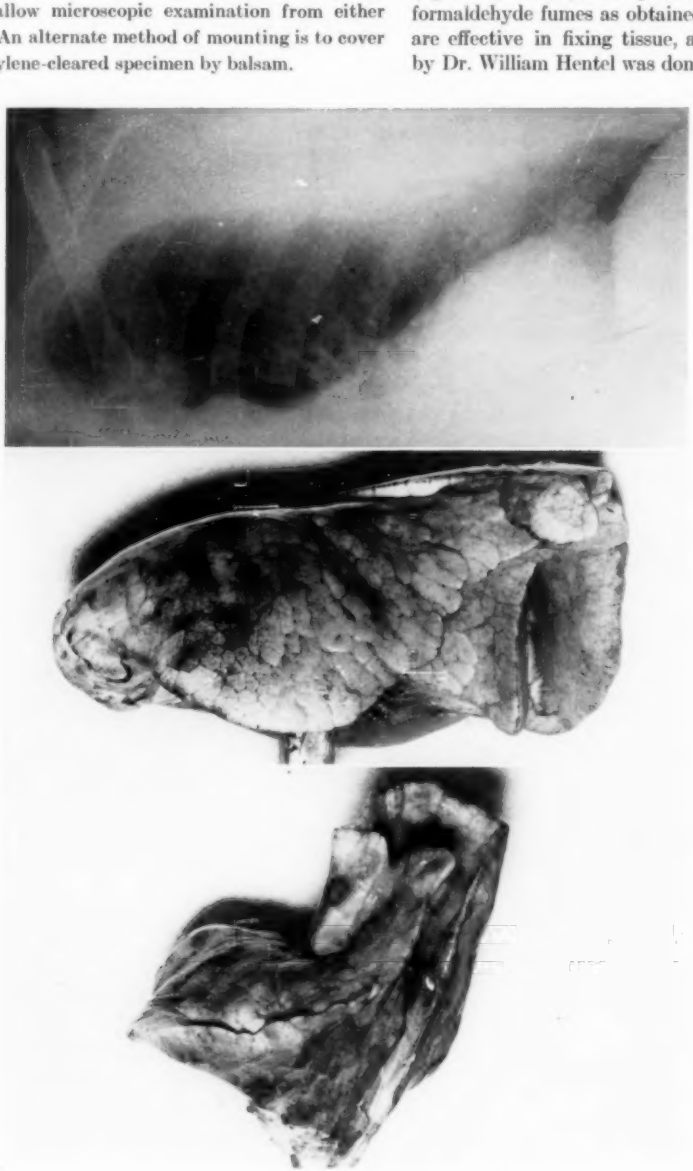

FIG. 3. Photographs and roentgenogram of left human lung. Photograph at left was taken before inflation and fixation; middle, after inflation and fixation. The roentgenogram was obtained prior to death.

of liver was suspended in an enclosed area with formaldehyde vapor entering under the same conditions as described for the lung. Another portion of liver was immersed in formalin for the same period. After twenty-four hours the liver exposed to formaldehyde fumes was fixed to a depth of 3.5 mm. from every exposed surface. The liver immersed in formalin was fixed to a depth of 5 mm.

A measure of the state of inflation was obtained by comparing a roentgenogram of the fixed lung (using the same distances as with a standard film) with an inspiratory chest film obtained ante mortem. A comparison of the gross specimen with the chest roentgenogram is demonstrated in figure 3. The projection areas in both instances were almost identical.

Consideration was given to the pressure applied to the lung. As there was a continual leak of air from the lung surface, the system had no static pressure which could be measured. By measuring the pressure in the inflating tube, the flow into the lung, the dimensions of the lung, and considering the lung as a porous body, the mean pressure applied to the lung was calculated. It was observed that by connecting the air source to the lung the maximal flow was reduced to 10 liters per minute. The usual flow to maintain the lung in an inspiratory state was 5 liters per minute. In these circumstances, the mean pressure applied to the lung was 1.35 to 18.2 cm. of water. At the beginning of inflation the maximal pressure applied to the lung was 36 cm. of water. These pressures are of the same magnitude as those applied to the lung during life. Artefacts in the form of local bulging at the surface could be produced only by pressures greatly exceeding this. When they occurred, they could be easily identified by the compression of underlying lung tissue and by the absence of strands of tissue connecting the underlying lung with the pleural surface. Such bulges were of limited extent so that most of the specimen was unaltered.

To measure the degree of shrinkage which occurred during the fixing process, horizontal and vertical circumferences were measured after inflation was first performed, and at daily intervals for seven days. These circumferences measured during the fixing process showed a decrease of 3 to 5 per cent.

No change of cellular or air-space size could be seen during the staining and mounting procedure. Cellular changes which occurred during the early fixing period could not be evaluated as re-

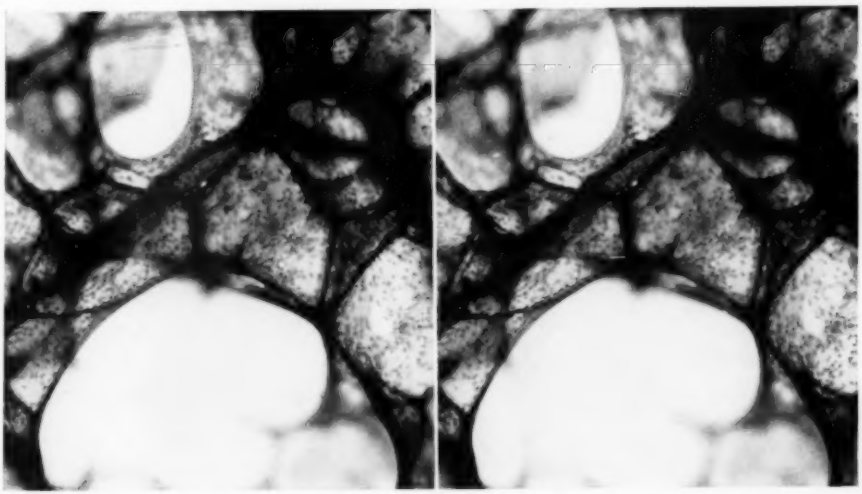

FIG. 4. Stereoscopic photomicrographs of air saccule showing arrangement of alveolar walls. Cellular structure and pores of Cohn are demonstrated. These photographs may be viewed by the use of conventional stereoscopic devices or directly by converging the eyes so that images are fused.

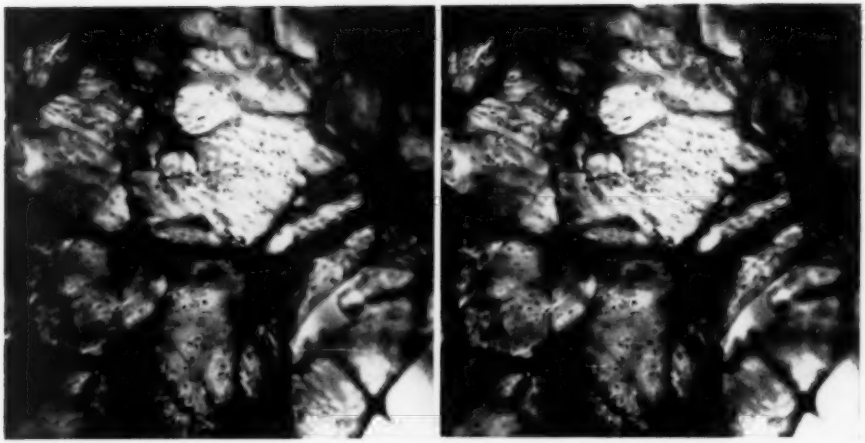

FIG. 5. Stereoscopic photomicrographs of alveolar walls demonstrating the configuration of capillaries.

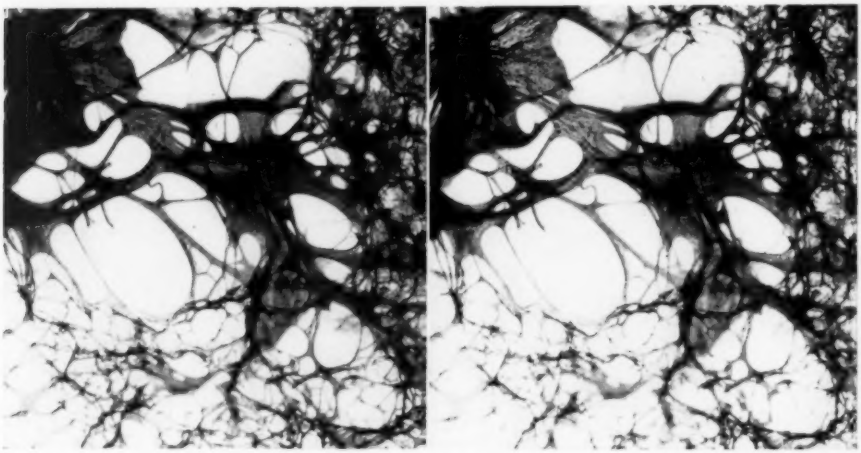

FIG. 6. Stereoscopic photomicrograph of human lung demonstrating a marked degree of emphysema. The degree of air space enlargement can be estimated by comparison with more normal lung seen at the extreme right of the figures.

moval of a portion of tissue allowed deflation of the lung.

The excellent cellular detail shown in stained sections provides evidence against artefacts of drying, shrinking, and fixing (figure 4). Various cell types can be identified, and proper staining defines the type of tissue present. As the tissue slices obtained are much thicker than those conventionally used, the three-dimensional relationships of anatomic structures are easily demonstrated by the use of stereoscopic microscopy. An air saccule with its arrangement of alveolar walls is demonstrated in figure 4. By varying the degree of magnification, the entire architecture from gross appearance to the details of cellular structure may be seen.

Examples of the type of tissues obtained are demonstrated in figures 5 to 7. Visualization of

these figures by stereoscopic means shows the three-dimensional relationships of the structures. Figure 5 was stained to demonstrate the configuration of capillaries within the alveolar walls. By contrast, the extensive alterations of emphysema are shown in figure 6. The presence of triangular and strandlike bridges of epithelial cells, some with identifiable capillaries, are seen to extend across large air spaces. A calcified nodule of tuberculosis surrounded by stellate bridges across enlarged air spaces is demonstrated in figure 7.

Specimens with subpleural blebs were examined by thick serial sections cut through the bleb

FIG. 7. Photograph of unstained human lung demonstrating a calcified nodule of tuberculosis with surrounding emphysema.

and its surrounding base. Destroyed areas similar to those pictured in figures 6 and 7 were found. Larger blebs removed at the time of surgery also demonstrated the presence of strandlike tissue connecting the under surface of the bleb with the underlying lung parenchyma.

Discussion

A method is presented of studying the morphology of the inflated lung in three dimensions. The lung is fixed in a simple manner so that it will retain an inspiratory position in a rigid state while being studied. The lung so obtained may be surveyed almost in its entirety by cutting whole lung sections of 1-cm. thickness. Such sections may be viewed by low-power (10 to 16 × enlargement) microscopy or stereoscopic microscopy. Large (35 by 60 mm.) selected areas of 0.3-mm. thickness may then be stained and examined in higher power by conventional or stereoscopic microscopy.

A historic account of the many means of inflating the lung is given by Miller (9). Numerous investigators have used air-dried specimens to study the geometry of air spaces. Studies of the inflating lung have been critical for those interested in the problem of collateral ventilation (10–16). Air-dried lung has been used to study emphysema by microradiology (17). An experimental method to obtain "solid-image" microscopy has been reported (18). However, it seems that the present method of morphologic examination is of such simplicity that it may be used in the routine study of pulmonary pathology.

Criticisms of the method of fixing include overdistention of lung with or without local bulging, inadequate fixation, artefacts of drying and shrinkage, and the necessity to alter conventional cutting and mounting techniques. Formaldehyde fume fixation compares favorably with formaldehyde immersion fixation. More elaborate techniques (19) might be used to evaluate fixation, but the ultimate test is in the tissue itself, and cellular detail is preserved by this method. The artefacts produced by drying should consist either of local shrinkage of cells or loss of volume of the entire organ. As cells were found to be intact and shrinkage is much less (3 to 5 per cent) as compared to immersion fixation in formalin (15 per cent), the method appears to have distinct advantages over conventional immersion fixation. Indeed, the continual application of air pressure at all times is believed essential to prevent distortion during fixation.

Revisions in cutting, mounting, and staining procedures are presented which provide excellent results. Selective strains may be used to good advantage. Ultimately, techniques may be devised which will give specimens of more uniform thickness. Injection of blood vessels by special means will demonstrate pulmonary vasculature more clearly.

The relatively recent identification of centrilobular emphysema (7, 20) is proof of the inadequacy of the immersion fixation technique to allow visualization of this lesion. The addition of three-dimensional studies in a routine manner allows greater precision and understanding of this lesion. The routine use of the method opens important avenues of approach to most pulmonary disease, whether it be tuberculosis, bronchitis, pneumoconiosis, pulmonary fibrosis, or emphysema. The central role of bronchiolar inflammation in diverse disease may be more clearly demonstrated. Correlation of these observations may result in greater unity between morphologic and physiologic studies (21).

Further potentialities of the method should now be considered. The freshly inflated lung may be allowed to deflate while it is being photographed by a movie camera. The rates of acceleration of different areas may be measured and related to underlying pathologic changes. After fixation the specimen is rigid, so that porosity of the whole lung and flow of air through porous plates of lung may be measured.

By the random placement of a marker upon the microscopic sections, area-to-volume ratios determined by the method of Chalkley and associates (22, 23) may be done. Not only the geometry of air spaces is to be studied, but also their variation in different parts of the lung and the changes accompanying various degrees of inflation.

Many aspects of emphysema may be more clearly defined. The type and distribution of lesions is to be related to aging, distribution of anthracotic pigment, alteration of pores of Kohn, vascular degeneration, and bronchiolar obstruction and inflammation. Attention is to be given to the pathogenesis of alveolar lateral wall destruction. The earliest physiologic changes are to be determined by measuring pulmonary function tests in patients with fatal nonpulmonary

disease and determining which of these patients had unrecognized early emphysema.

The further elucidation of many aspects of pulmonary disease should follow the experimental production of pulmonary lesions. This method is particularly well suited to survey the whole lungs of animals, and so may allow anatomic evaluation of experimental procedures.

Summary

A method of fume fixation of lung is described which provides an inflated, rigid specimen and allows structure of whole lungs to be surveyed.

Revisions of conventional cutting, staining, and mounting procedures provide large thick specimens which allow the application of a wide variety of staining techniques. Three-dimensional structure of either the stained or unstained specimen is demonstrated by stereoscopic microscopy so that relationships present during life are more closely approximated.

Sumario

La Estructura Pulmonar en Tres Dimensiones después de la Inflación y Fijación con Vapor de Formaldehido

El método aqui descrito de fijación del pulmón con vapores de formaldehido produce un ejemplar rígido e inflado y permite estudiar la estructura de los pulmones íntegros.

Las revisiones de los procedimientos convencionales de resección, coloración y montaje suministran grandes y gruesos ejemplares que permiten la aplicación de una gran variedad de técnicas de coloración. La estructura en tres dimensiones del ejemplar ya teñido o no queda revelada por la microscopía estereoscópica de modo que semeja mejor las relaciones que existen durante la vida.

Resume

La structure à trois dimensions du poumon après inflation et fixation au moyen de vapeurs de formaldehyde

Description d'une méthode de fixation des gaz du poumon qui procure une pièce rigide et distendue permettant d'examiner la structure du poumon dans son ensemble.

La révision des procédés conventionnels de section, de coloration et de montage, procure des spécimens épais de grandes dimensions permettant l'application de techniques de coloration très variées. La structure à trois dimensions des spécimens colorés, ou non, est révélée par la microscopie stéréoscopique, ce qui facilite une évaluation aussi proche que possible à l'égard des rapports existants durant la vie.

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THE EVOLUTION AND EARLY RESULTS OF TRACHEAL FENESTRATION[1]

E. E. ROCKEY, S. A. THOMPSON, AND C. F. BLAZSIK

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INTRODUCTION

Tracheal fenestration is an airtight, leakproof, skin-lined tracheocutaneous opening, created for the purpose of repeated tracheobronchial aspirations or self aspirations and medications (1–4). The purpose of this paper is to present the evolution of tracheal fenestration and the experiences and results with 17 consecutive cases.

General Review

At the end of 1954, one of the writers (EER) started a series of animal experiments for the purpose of developing an operation which had the advantages, without the disadvantages, of conventional tracheostomy. The idea for such a procedure in the management of pulmonary suppuration in inoperable cases was suggested by Drs. Edgar Mayer and Israel Rappaport. Through the efforts of Dr. Mayer, funds, animals, and space for the basic experiments were provided for Dr. E. E. Rockey at the New York University-Bellevue Medical Center. These experiments were described in a report under the title of "Tracheal Fenestration" in August, 1956 (1). The initial animal experiments were concluded by June, 1955. At this time, the search for a suitable case of pulmonary suppuration began, with the hope of accomplishing at least some palliative relief. The active participation of Dr. S. A. Thompson and the interest of Dr. I. G. Epstein of the New York Medical College-Metropolitan Medical Center led to the first patient on whom this operation was performed by the writers at the Metropolitan Hospital, New York City, on January 30, 1956.

The operation could not be completed because of technical problems. The patient was left with a gaping tracheocutaneous opening which, nevertheless, allowed repeated tracheobronchial aspirations and medications. In the meantime, additional animal experimentation led to an improved technique, making the transference of the operation from the animal to the human possible. Accordingly, at a second stage, the operation was successfully completed at the Metropolitan Hospital on April 4, 1956.

The palliation obtained by repeated tracheobronchial aspirations and instillation of cycloserine in this 34-year-old moribund, Negro male with biilateral, far advanced active pulmonary tuberculosis was striking (3). The ill effects of the immediate postoperative use of the skin tract soon became obvious. This fine plastic procedure was destroyed to a great extent by the repeated daily catheter insertions for aspirations. The need for further revision was apparent. These studies were conducted at the laboratories of the New York Medical College and led to the present operation (*see* figure 2).

This consists of a tracheal fenestration and the simultaneous performance of a temporary tracheotomy below the level of the fenestration. Until the fenestration heals, the tracheotomy is utilized for the catheter insertions. This is accomplished in three to four weeks; then the tracheotomy cannula is removed, its site allowed to heal, and the function of the fenestration begins. The combined procedure was performed for the first time by the writers at the Flower and Fifth Avenue Hospitals on June 22, 1956, on a 5½-year-old white boy with cystic fibrosis of the pancreas with advanced pulmonary suppuration. The child gained definite palliative benefit from this procedure (3).

The third case, that of a 32-year-old Negro male, suffering from suppurative disease of the lungs and marked emphysema, was submitted to the combined operation at Metropolitan Hospital on February 25, 1957. This patient also gained definite palliative benefit from the procedure (3).

A further advance in the clinical application of tracheal fenestration began in October, 1957, at St. Anthony's Hospital. A bedridden patient with so-called "dry" emphysema was suggested for tracheal fenestration after all available medical treatment had failed. The 52-year-old, dyspneic, cyanotic, white male had no cough and little or no expectoration. His breath sounds were depressed to absent, and the lower intercostal spaces were retracted in the inspiratory phase of respiration, suggesting peripheral bronchial and bronchiolar occlusion. The initial opinion of the conference was that no further help could be offered for this desperately ill man who had already been submitted to all the accepted forms of conventional therapy. The final decision for operation was made by Dr. Charles F. Blazsik.

[1] From the New York Medical College, Metropolitan Medical Center, New York, New York; and St. Anthony's Hospital, Woodhaven, Long Island, New York.

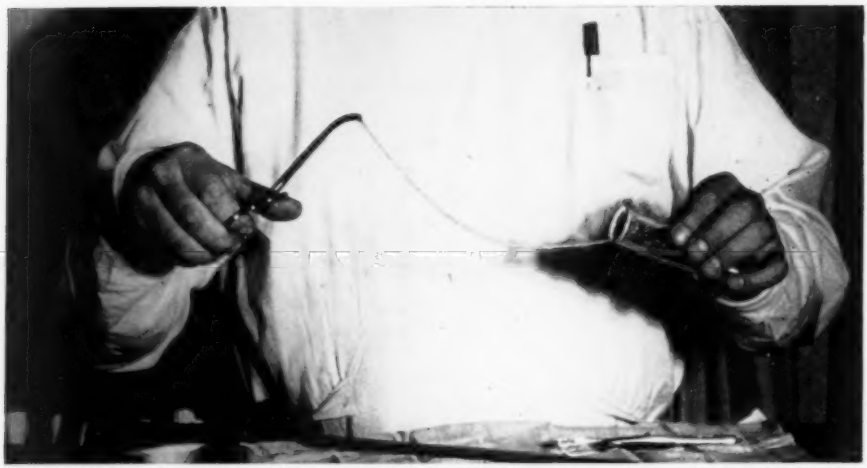

FIG. 1. Whitish, tenacious, stringy mucoid material aspirated from the tracheobronchial tree of a so-called "dry" case of emphysema (*Case 4*).

FIG. 2. Tracheal fenestration seven weeks after operation (*Case 7*); the fenestration is healed. The site of the previous tracheotomy cannula is covered with a small dressing.

The findings of obstructive inspiration on inspection and on physical examination mentioned above suggested that the major disability in advanced emphysema cases originates in the peripheral bronchi and bronchioles, and is essentially a secretional occlusion. Subsequently, this theory was substantiated, as described below, and has served as a guide in the selection of later cases. The removal of large quantities of whitish, tenacious, stringy, mucoid material (figure 1) from the tracheobronchial tree by repeated aspirations has led to rehabilitation of this patient, who, before fenestration, was a respiratory cripple (4). The striking improvement in this patient and his eventual return to full-time work is responsible for the rapid succession of the cases now reported.

CLINICAL SERIES

The present study deals with 17 consecutive cases of patients[2] operated upon at Metropolitan Hospital, Flower and Fifth Avenue Hospitals, St. Anthony's Hospital, and Manhattan General Hospital Chest Pavilion from January, 1956, to August, 1958, inclusive (table 1). Four of the patients were Negro and 13 were white. Sixteen of the series were males and one was female. The age of the patients ranged from 5½ to 82 years, with a mean age of 54.7 years. The preoperative diagnoses were as follows: far advanced, bilateral, active pulmonary tuberculosis, one patient; cystic fibrosis with advanced pulmonary suppuration, one patient; active, far advanced silicotuberculosis, one patient; advanced emphysema with chronic suppurative bronchitis or bronchiectasis, 7 patients; and advanced so-called "dry" emphysema, 7 patients. One of the latter group

[2] Included 3 patients reported in reference (3) and 4 patients reported in reference (4).

TABLE 1

A Chronologic List of 17 Cases Consecutively Submitted to Tracheal Fenestration

	Name	Color	Age	Sex	Name of Hospital	Diagnosis	Date of Operation	Medical Supervision	Results
			years						
1	F.V.	N	34	M	Met.*	Bilateral far advanced	1–30–56	Epstein	Palliation, died
2	J.C.	W	51½	M	F.F.A.H.†	Active pulmonary tuberculosis Cystic fibrosis	4–5–56 6–22–56	Slobody and Wasserman	Palliation, died
3	J.H.	N	32	M	Met.	Emphysema with infection	2–25–57	Epstein	Palliation, died
4	G.M.	W	52	M	St. Anthony's‡§	Emphysema, "dry"	10–28–57 11–4–57	Blazsik	Marked rehabilitation
5	W.S.	W	62	M	St. Anthony's	Emphysema, "dry"	12–2–57	Blazsik	Palliation, died
6	M.M.	N	63	M	Man. Genl.§	Emphysema with infection	12–6–57	Edlin	Rehabilitation, died
7	E.K.	W	63	F	F.F.A.H.	Emphysema with infection	3–21–58	Schochet	Marked rehabilitation
8	J.V.	W	61	M	Man. Genl.	Emphysema with infection	4–1–58 7–15–58	Snieska	Rehabilitation
9	V.R.	W	65	M	Met.	Emphysema, "dry"	4–15–58	Epstein	Rehabilitation
10	B.C.	W	67	M	St. Anthony's	Emphysema with active silicotuberculosis	4–23–58 5–29–58	Blazsik	Marked palliation
11	N.P.	N	65	M	F.F.A.H.	Emphysema, "dry" with cor pulmonale in failure	6–3–58	Boyd and Diamond	Palliation, died
12	S.B.	W	50	M	F.F.A.H.	Emphysema with infection and cor pulmonale in failure	6–5–58 11–6–58	Beck	Marked palliation
13	L.F.	W	82	M	St. Anthony's	Emphysema, "dry"	6–20–58	Blazsik	Marked rehabilitation
14	M.M.	W	55	M	F.F.A.H.	Emphysema with infection and cor pulmonale in failure	6–23–58	Mayer and Rappaport	Marked palliation, died
15	J.C.	W	58	M	F.F.A.H.	Emphysema, "dry" and carcinoma of right lung	6–26–58	Mayer and Rappaport	Palliation, died
16	J.S.	W	53	M	F.F.A.H.	Emphysema, "dry" with arteriosclerotic heart disease (in and out of failure)	7–23–58	Gottesman	Marked palliation
17	S.P.	W	62	M	St. Anthony's	Emphysema with infection	7–30–58 8–1–58	Blazsik	Marked palliation

* Metropolitan Hospital
† Flower and Fifth Avenue Hospitals
‡ St. Anthony's Hospital
§ Manhattan General Hospital

also had bronchogenic carcinoma of the left lung. Fifteen of the 17 patients were bedridden and required almost continuous or intermittent oxygen therapy. Only 2 of the patients were able to walk approximately 20 to 30 steps before getting into great respiratory distress. Two of the patients also had cor pulmonale and were known to have been in cardiac failure for more than five years. One of the patients had arteriosclerotic heart disease with coronary arterial insufficiency in addition to advanced emphysema, and one had advanced emphysema with arteriosclerotic heart disease and cor pulmonale. The 2 last-named patients were in and out of failure for at least three years prior to their tracheal fenestration.

Results

Palliation: Palliation occurred in 6 patients. This term is used to designate patients who were made more comfortable than they had been prior to operation. Their exercise tolerance increased, their respiratory rate and pulse rate decreased, and their color improved. Although these patients were helped, eventually they died because of their advanced pulmonary disease (*Cases 1, 2, 3,* and *5*), cardiovascular disease (*Case 11*), and other diseases such as carcinoma (*Case 15*).

Marked palliation: Marked palliation occurred in 5 patients. This term indicates patients who showed improvement of pulmonary ventilation and exercise tolerance and who no longer required oxygen. The over-all progress has been slower than usual, due to advanced cor pulmonale (*Cases 12* and *14*), arteriosclerotic heart disease (*Case 16*), or active pulmonary tuberculosis (*Case 10*). One of these patients died of an unrelated condition (duodenal ulcer with hemorrhage).

Rehabilitation: Rehabilitation was accomplished in 3 patients. By rehabilitation is meant the improvement of ventilation and exercise tolerance sufficiently not only to enable patients to get around and attend to their personal needs without oxygen or the assistance of others, but to walk several blocks without respiratory embarrassment. These patients have a chance to undergo even more improvement. They are about to be discharged or already have been discharged from the hospital. One of these patients died of right spontaneous tension pneumothorax.

Marked rehabilitation: The classification *marked rehabilitation* has been applied to 3 patients. Their condition improved sufficiently to allow return to gainful occupations, if that were desired by the patients.

TABLE 2

A. List of the Results

Palliation*	6
Marked palliation	5
Rehabilitation	3
Marked rehabilitation	3
Total	17

* For definition of categories see text.

TABLE 3

Analysis of Deaths

Far advanced, active pulmonary tuberculosis	1
Cystic fibrosis of the pancreas with advanced suppurative disease of the lungs	1
Advanced emphysema with suppurative disease of the lungs	2
Cerebrovascular accident	1
Carcinoma of the lung	1
Spontaneous tension pneumothorax	1
Hemorrhage due to duodenal ulcer	1
Total	8

Present status: Palliation was accomplished in 6 patients and marked palliation in 5 patients (table 2). Four of the latter group are alive. Rehabilitation was accomplished in 3 of the patients. Two of this group are alive. Marked rehabilitation was accomplished in 3 patients. All of these are alive. There were no operative mortalities, and no patient failed to receive some improvement. Nine of 17 patients are alive. Analysis of the deaths (table 3) indicates that one patient died of bilateral, far advanced, active pulmonary tuberculosis; one died of cystic fibrosis of the pancreas with far advanced pulmonary suppuration; 2 died of far advanced emphysema; one, of cerebrovascular accident; one, of carcinoma of the lung; one, of spontaneous tension pneumothorax; and, finally, one, of massive hemorrhage from a large duodenal ulcer.

Discussion

The basic experiments leading to the development of tracheal fenestration were started with the idea of producing a palliative operation to be

used in the treatment of advanced and inoperable cases of pulmonary suppuration. The idea of applying this operative procedure to other conditions came as experience increased.

The first patient on whom this operation was performed was a case of far advanced, active, pulmonary tuberculosis with excessive drainage. The observations made in this case encouraged the employment of tracheal fenestration in other cases of active tuberculosis for which other forms of therapy were unsatisfactory, or cases which were more or less abandoned. The easy elimination of the thick, tenacious material from the tracheobronchial tree of the second patient with cystic fibrosis of the pancreas with advanced pulmonary suppuration convinced the writers that tracheal fenestration offers a new hope for these unfortunate children with otherwise fatal termination. The wider application of this operative procedure was begun with the first case of advanced emphysema with chronic suppurative bronchitis (*Case 3*) and with the first case of so-called "dry" emphysema (*Case 4*). Fifteen of the 17 patients reported here had advanced emphysema with marked respiratory disability. Seven of them had so called "dry" emphysema, and 8 had emphysema with chronic suppurative bronchitis.

The patients with the so-called "dry" emphysema had minimal or no cough and virtually no expectoration. They were all cyanotic and used their accessory respiratory muscles during inspiration even at rest. They all had marked inspiratory retraction of the lower intercostal spaces, and the chest wall expansion was limited. Auscultation revealed depressed to absent breath sounds in all. These findings suggested peripheral bronchial and bronchiolar obstruction by retained secretions. This idea was well substantiated by the removal of the secretions by repeated tracheobronchial aspirations after tracheal fenestration was performed. In the operating room, a considerable amount of thick, tenacious, stringy, whitish material was eliminated by tracheobronchial aspirations from all of these patients. Within six to eight hours of the operation, by which time several aspirating sessions were applied, the breath sounds were more clearly audible, the extent of the chest wall expansion increased. These findings give further proof of the secretory occlusion theory.

Improvement became more and more pronounced and the exercise tolerance of the patients increased during the ensuing weeks and months. Within the first seven to ten days after operation, the amount of tracheobronchial secretion is abundant and the patients need to be aspirated frequently (fifteen-minute to hourly intervals). After this period elapses, the amount of secretion removed per day decreases, and the need for tracheobronchial aspirations becomes less and less. Most of these patients required only one to four aspirating sessions daily by the time they were discharged from the hospital. By this time the evidence of bronchial occlusion on inspection and auscultation had disappeared. The improvement in the ventilation of these patients began in the operating room and the progress has been parallel with the diligence of the aspirations performed.

It is believed that the aforementioned facts indicate that the disability of these patients originated in the progressive peripheral bronchial and bronchiolar secretional occlusion which led to a continuing decrease in the amount of respiratory surface. By clearing not only the central, but—even more—the peripheral airways, the respiratory surface was increased, which in turn explains the striking clinical improvement of these patients. Undoubtedly, other factors are involved in the improvement of these patients, but the removal of the secretional occlusion appeared to be the major one.

Eight patients had advanced emphysema with chronic suppurative bronchitis. These patients complained of excessive cough with purulent and mucopurulent expectoration. They all were cyanotic and used their accessory respiratory muscles even at rest. They also had limited chest wall expansion and inspiratory retraction of the lower intercostal spaces. The breath sounds were depressed, and in all instances moist rales and occasionally even wheezes could be heard. Following tracheal fenestration and repeated vigorous tracheobronchial aspirations, the chest wall expansion increased, the breath sounds became more audible, the rales decreased and eventually disappeared. Along with the change in the physical findings, the ventilation of the patient improved and the exercise tolerance increased. After tracheal fenestration, the purulent or mucopurulent bronchial secretion gradually changed to a whitish, tenacious, sticky, and stringy material similar to the substance obtained at the

operation of the so called "dry" emphysema cases. Obviously the situation here represented the same condition complicated by bronchial infection. The latter is controlled by the drainage provided by the tracheobronchial aspirations.

There is considerable difference in the clinical course of the patients with obvious cor pulmonale in failure or arteriosclerotic heart disease in failure as compared with the patients with advanced emphysema without evidence of heart disease. The progress of the 3 patients with advanced cor pulmonale in failure and of the one patient with advanced arteriosclerotic heart disease in failure in the series presented here was much slower than the progress of the rest of the patients. The former patients needed a great deal more nursing care, and it usually required several weeks before they were able to aspirate their own tracheobronchial tree. By contrast, the patients without heart disease were able to start self aspirations on the second or third day after the operation and, within one week, were quite proficient in taking care of themselves. The improvement in ventilation of the patients with cor pulmonale in failure was striking even though the cardiac status remained apparently unchanged. The reason for treating these patients by means of tracheal fenestration was to make an effort to control the pulmonary disease which led to the cor pulmonale. In addition to arresting the progress of the cor pulmonale, it was believed that the re-aeration and the elimination of the infection from the peripheral airways might even lead to re-canalization of the compressed or obliterated peripheral pulmonary vessels, with subsequent lowering of the pulmonary hypertension. It is hoped that tracheal fenestration, by providing better ventilation, will keep these patients alive long enough for such changes to take place.

The indications for tracheal fenestration presently used by the writers are: advanced cases of emphysema with or without suppuration and with marked respiratory disability; excessive suppurative disease of the lungs when excisional surgery is contraindicated; fibrocystic disease of the pancreas with excessive pulmonary suppuration; and far advanced cases of pulmonary tuberculosis, when no other form of therapy can be offered.

It is believed that additional indications will become manifest as experience increases; for example, the procedure might have some use in the bulbar type of poliomyelitis requiring a mechanical respirator.

Summary

Experience with 17 consecutive cases of severe chronic respiratory disease treated with tracheal fenestration and repeated tracheobronchial aspirations and self aspirations is reported. Six of these patients experienced some palliation and 5 had marked palliative relief. Three patients were rehabilitated to a degree, and 3 gained the status of "marked rehabilitation." Nine of the 17 patients are alive. There were no operative deaths in the series. The observations confirm the initial impression that the major source of the disability in advanced cases of emphysema, including the so-called "dry" emphysema, is secretional occlusion of the peripheral bronchi and bronchioles rather than bronchial spasm.

Sumario

La Evolución y los Resultados Tempranos de la Fenestración Traqueal

Repásase lo observado en 17 casos consecutivos de grave enfermedad respiratoria crónica tratada con la fenestración traqueal y repetidas aspiraciones tráqueobronquiales y autoaspiraciones. Seis de estos enfermos obtuvieron alguna paliación y 5 decidido alivio paliativo. Tres enfermos se rehabilitaron hasta cierto punto y 3 captaron el estado de "notable rehabilitación." Nueve de los 17 enfermos están vivos. No hubo mortalidad operatoria en la serie. Las observaciones realizadas confirman la impresión inicial de que la causa principal de la incapacidad en los casos avanzados de enfisema, comprendiendo el llamado "seco," es la oclusión secrecional de los bronquios y los bronquiolos periféricos más bien que el espasmo bronquial.

Resume

Evolution et résultats précoces de la fenestration trachéale

Rapport sur une série de 17 cas d'affections respiratoires sévères chroniques ayant été traités par la fenestration trachéale, les aspirations trachéo-bronchiques et les self-aspirations répétées. Une certaine atténuation des symptômes s'est produite chez 6 malades et un effet palliatif marqué a été obtenu chez 5. La réadaptation de 3 des malades a été réalisée à un certain degré et 3 ont pu être classifiés "réadaptation marquée." Parmi ces 17 malades, 9 sont vivants. Il ne s'est

produit aucun décès chirurgical dans cette série. Les observations confirment l'impression initiale que la source majeure d'invalidité dans les cas avancés d'emphysème, y compris le soi-disant emphysème "sec", est l'occlusion des bronches et bronchioles périphériques par les sécrétions, plutôt que par un spasme bronchique.

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PULMONARY RESECTION FOR DRUG-RESISTANT CAVITARY TUBERCULOSIS UTILIZING ANCILLARY DRUGS[1,2]

WATTS R. WEBB

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Introduction

The patient with persistent cavitary tuberculosis with tubercle bacilli in the sputum resistant to the major antituberculous drugs constitutes one of the most difficult problems facing the phthisiologist today. Further medical therapy offers little except to prolong the state of chronicity. In addition, the surgical experiences of Coleman, Holland, Lees, Hughes, Robinson, and Wareham, and their associates (1–6) have documented the marked increase in both early and late complications following surgery in these patients with drug-resistant infections. Recently the patients have been subjected to resection, utilizing other antituberculous drugs for temporary protection, such as viomycin (7), pyrazinamide (8), viomycin with oxytetracycline (2), and viomycin with pyrazinamide (9), although the number of such cases reported have been insufficient for full evaluation. Pecora and Yegian (10) correlated resistance studies with the clinical results, which suggests that the mere presence of resistant organisms is of little importance provided other effective drugs are available. One effective drug, either streptomycin, isoniazid, or pyrazinamide, seemed to provide protection against the early postoperative complications, although viomycin by itself did not. Two or more drugs were better than a single drug in preventing late complications. A previous report (11) related the present writer's experiences with 35 resections in 32 such patients, utilizing viomycin with thiazosulfone[3] or pyrazinamide for antimicrobial therapy during the surgical period. This experience has now been extended to 68 resections in 62 patients, who have been followed from four to fifty-six months. An analysis of this experience comprises the present report.

Composition of Series

All 62 patients (table 1) had open tuberculous lesions with tubercle bacilli in the sputum of proved or presumptive resistance to streptomycin, isoniazid, and PAS. In every case, the presence of tubercle bacilli in sputum or gastric washings had been demonstrated within the two-month period immediately prior to surgery. Tubercle bacilli were classified as resistant if growth was not inhibited by streptomycin or PAS concentrations of 10 γ per ml. or by isoniazid concentrations of 1 γ per ml. Patients were classified as having presumptively resistant infections if the bacilli were not cultured but could be seen on microscopy and if one or more cavities had remained open despite chemotherapy for at least one year. In the study by Decker, Raleigh, and Welles (12), if the cavity lesions remained open during the first year of chemotherapy 80 per cent of the patients were discharging drug-resistant bacilli. This percentage quickly increased as the time of chemotherapy was extended further. Included in the present series are a few patients whose strains of tubercle bacilli were resistant to only two of the three major antituberculous drugs, but drug toxicity prevented utilization of the third drug.

Each patient was given viomycin and pyrazinamide for a two-week period preoperatively and this was continued for a period of approximately three months postoperatively. Six of the patients included in the initial report received thiazosulfone which has not been used since the more potent pyrazinamide became available. The usual dosages administered were viomycin, 2 gm. twice a week with either 1 gm. of the sulfone four times daily or 500 mg. of pyrazinamide four times daily. Streptomycin, isoniazid, and PAS were continued for whatever value they might possess, in the belief that many of the bacilli in areas outside the cavity might be susceptible to their action.

Only 5 patients had been receiving chemotherapy less than twelve months, and in these cases the bacilli were proved resistant by *in vitro* tests. The remainder of the patients had been receiving chemotherapy for periods of one to seven years at the time of pulmonary resection. Forty-three of the patients were re-treatment cases, having had interrupted courses with two or all three of these drugs.

The duration of total illness ranged up to seven-

[1] From the departments of surgery, Mississippi State Sanatorium and the University of Mississippi Medical Center, Jackson, Mississippi.

[2] Presented at the annual meeting of the Southern Trudeau Society, Richmond, Virginia, October 2, 1958.

[3] Promizole®.

TABLE 1
PATIENTS COMPRISING PRESENT REPORT

White		Negro	
Male	Female	Male	Female
25	15	11	11

TABLE 2
DURATION OF DISEASE

Years	< 1	1	2	3	4	5	6–10	11–17
Patients....	1	11	8	10	9	4	16	3

TABLE 3
AGES

Years	18	20–29	30–39	40–49	50–59	60–69
Patients....	5	8	17	20	10	2

TABLE 4
ROENTGENOGRAPHIC CLASSIFICATION OF DISEASE

	Ipsilateral	Contralateral
Minimal	0	40
Moderate	21	16
Far advanced	41	6
Cavitary	62	19

teen years, and only 20 of these patients had had tuberculous disease for less than three years (table 2). The patients varied in age from eighteen to sixty-five years, 32 of the patients being more than 40 (table 3). Twenty of the patients had had previous collapse therapy: pneumoperitoneum in 14; pneumothorax in 9; thoracoplasty in 10, among whom 3 had bilateral thoracoplasties.

Classification of the disease by roentgenography on admission (table 4) showed that 41 had far advanced disease and 21 had moderately advanced tuberculosis. There were no minimal lesions. The contralateral lung was considered normal or only minimally diseased in only 40 patients, while 19 patients showed definite contralateral cavitation.

The resections included 16 pneumonectomies, 24 lobectomies, 8 lobectomies plus segmental procedures, and 20 segmental resections. These operations presented marked technical difficulties, as dense adhesions, hilar fibrosis, and multiple enlarged adherent lymph nodes were common findings. Significant hemorrhage occurred in 4 cases of this series due to injury to the vascular structures, but it was possible in every case to control the hemorrhage and complete the contemplated procedure.

OBSERVATIONS

Deaths

There were 5 deaths in the series (table 5). All occurred early, but none were related to tuberculous disease or to the fact that the infections were resistant to the major antituberculous drugs. Two patients died of pulmonary emboli, one on the operating table, and one died on the twentieth postoperative day. One died of pulmonary insufficiency six days postoperatively, and a fourth died of pulmonary edema the day after surgery. The fifth patient died the day of surgery from a hemolytic blood transfusion reaction.

There was one transient cardiac arrest during operation which responded immediately to therapy, and the operation was completed without sequelae. There were four postoperative hemothoraces which cleared with enzymatic debridement.

Eleven postoperative tracheostomies were performed in this group. Although it is the practice on this service to resort to tracheostomy on minimal indications for retained secretions or respiratory insufficiency, this is a far greater incidence than in most other patients who undergo surgery for tuberculosis. Six of these patients showed definite respiratory insufficiency and 3 required artificial ventilation postoperatively for periods up to seven days.

Drug Toxicity

In 4 patients viomycin was discontinued because of persistent rash and itching, and 4 suffered definite anaphylactoid reactions, with urticaria, dyspnea, facial edema, and hypotension. Treatment with intravenous fluids and anti-

TABLE 5
COMPLICATIONS

Deaths	5
Cardiac arrest	1
Hemothorax	4
Bronchopleural fistula	4
Pulmonary insufficiency	6
Tracheostomy	11

histaminics was effective in each case. Liver function tests became abnormal in one patient receiving pyrazinamide who did not demonstrate clinical symptoms, and the values for liver function returned to normal after discontinuance of the drug. Sulfone therapy was discontinued, as previously reported, in one instance because of a recurrent rash.

Follow-up Observations

The patients have been followed from four to fifty-six months (table 6). There have been 2 roentgenographic relapses, 5 bacteriologic relapses, and 7 relapses of both types. All but 5 of the patients now have inactive tuberculosis following further drugs or surgical therapy. Both of the roentgenographic relapses were contralateral reactivations at the site of previous disease, and these have been controlled by further drug therapy, and in one patient by a complementary pneumothorax. One of the patients with an early bacteriologic relapse attained a noninfectious status on further drug therapy, only to again discharge tubercle bacilli some two years later, although the roentgenograms revealed no evidence of disease activity. The other patients with bacteriologic relapses attained and maintained noninfectious status after further antimicrobial treatment. Four of the combined relapses have been due to bronchopleural fistulas, one manifesting itself late and the others early. The early bronchopleural fistulas, two of which appeared after right upper lobectomies and the other after a left pneumonectomy for progressive caseous pneumonia, have been closed with thoracoplasties and silver nitrate cauterizations. The late bronchopleural fistula developed from the stump of the superior division of the left lower lobe which had been resected with the left upper lobe. The peribronchial empyema persists and the sputum remains positive for tubercle bacilli.

TABLE 6
RELAPSES

	Roentgenographic	Bacteriologic	Combined
Early	1	2*	4**
Late	1	3	3**

* One still active.
** Two still active.

TABLE 7
PRESENT STATUS

	Patients	Per Cent
Dead	5	8.1
Living with active tuberculosis	5	8.1
Living and well	51	83.8

One late relapse occurred in one of the 6 patients treated with viomycin and thiazosulfone. Bilateral plombage thoracoplasties had failed to accomplish permanent sputum "conversion," and the superior division of the left lower lobe had been resected for a residual cavity. Eight months later the sputum again became positive for tubercle bacilli, and roentgenography revealed a cavitary reactivation in the right lower lobe, which subsequently has been resected, with good result. Another patient, W.B.M., previously reported, had the superior division of the right lower lobe resected after failure of a plombage thoracoplasty. After discharge, reactivation of the lesion occurred in the right upper lobe, and subsequent right upper lobectomy elsewhere resulted in a bronchopleural fistula with the continuing discharge of tubercle bacilli in the sputum.

Thus, 5 of the 62 patients have died and 14 have shown subsequent tuberculous disease, although 9 of the latter are now clinically well (table 7). Interpreted in a different fashion, 69.4 per cent of the patients have been consistently well since surgery, and an additional 14.5 per cent have been salvaged by further surgery or drug therapy.

DISCUSSION

The results appear gratifying in view of the nature of the disease in these patients. The operative mortality has been high, but no deaths were due directly to tuberculosis. There were 7 early tuberculous complications in the three-month period following surgery while the ancillary drugs were being given. Subsequent to that time, there have been 7 additional tuberculous complications, 5 of which have responded to additional drugs or to surgery. In view of the extensive and bilateral nature of the disease in many of these patients, the long-term results will undoubtedly become poorer as time goes on.

Figures illustrating the nature of the disease

in several cases, together with the results of the therapy, will be shown at the end of the article.

Contrary to the experience of some (2), the writer has not noted undue complications following segmental resections, which actually have resulted in far better results than either the lobectomies or pneumonectomies. Only one of the segmental resections has shown a tuberculous complication, although there have been tuberculous complications in 3 of the 8 patients with combined lobectomies and segmental resections. Accordingly, it is believed that one should be governed in his choice of procedure solely by the extent of disease and not by the drug status, so long as some potentially effective drug regimen is available.

The results in this group resected under the protection of viomycin and pyrazinamide are compared in tables 8 and 9 with a similar group collected from the data of the Veterans Administration-Armed Forces cooperative studies (13). The latter group of patients had pulmonary resections between July, 1955, and June, 1956. All had bacilli that were demonstrably resistant to streptomycin, but no definite information is available concerning the status of the patient-strains with respect to isoniazid or to PAS. The resections presumably were performed without benefit of additional drugs, and the marked difference in results is apparent.

While few patients now reach this chronic active state under sanatorium care, the increasing use of drugs in home treatment without the availability of frequent surgical evaluation has allowed many patients to miss the time for optimal surgery. In the present series, 40 of the 62 patients had drug-resistant infections before admission to the sanatorium. This danger has been emphasized by Allen (14), who also found that only 2 per cent of the patients became chronic cases when properly and immediately treated in the sanatorium with combined medical and surgical therapy. An additional 3 per cent died of tuberculosis, and 3 per cent died of other diseases.

TABLE 8

COLLECTED CASES

	VA-AF*	Present Series
Pneumonectomy	15	16
Lobe	36	32
Segment	20	20
Total	72	68

* Veterans Administration—Armed Forces (13).

TABLE 9

COMPARSION OF COMPLICATIONS

	VA-AF*		Present Series	
	Number	Per Cent	Number	Per Cent
Deaths	8	11	5	8
Fistula	17	24	4	7
Empyema	16	22	4	7
Tubercle bacilli in sputum	39	54	11	18

* Veterans Administration—Armed Forces (13).

SUMMARY

An analysis is presented of the temporary protection of viomycin combined with thiazosulfone or pyrazinamide in 68 pulmonary resections in 62 tuberculous patients who have been followed from four to fifty-six months. Of this group, 83.8 per cent are now clinically well, although only 69 per cent have been free of tuberculous complications since surgery. In this group of patients, the mechanical factors that would prevent cavity closure, regardless of the duration or nature of medical therapy, continue to be impressive. Viomycin along with pyrazinamide appears to be of value as temporary protection for excisional therapy of tuberculous lesions in which the bacilli are resistant to the major antituberculous drugs.

SUMARIO

La Resección Pulmonar por Tuberculosis Resistente, Positiva, Abierta, Utilizando Drogas Coadyuvantes

Preséntase un análisis de la protección temporal que otorgó la viomicina combinada con la tiazosulfona o la pirazinamida en 68 resecciones en 62 enfermos mantenidos en observación durante cuatro a cincuenta y seis meses. De este grupo, 83.8 por ciento se hallan actualmente clínicamente bien, aunque sólo 69 por ciento han permanecido exentos de complicaciones tuberculosas consecutivamente a la cirugía.

En este grupo de enfermos, retienen su im-

portancia los factores mecánicos que impedirían el cierre de las cavernas, independientemente de la duración o la naturaleza de la terapéutica médica.

Resume

Résection pulmonaire pour tuberculose cavitaire ouverte bacillifère et résistante avec traitement par des médicaments ancillaires

Présentation d'une analyse concernant la protection temporaire offerte par la viomycine associée à la thiazosulfone ou la pyrazinamide dans 68 résections effectuées chez 62 patients, qui ont été observés pendant quatre à cinquante-six mois. Dans ce groupe, 83,8% sont maintenant en bon état clinique, bien que 69% seulement d'entre eux aient été indemnes de complications tuberculeuses depuis l'intervention.

Dans ce groupe de malades, l'importance des facteurs mécaniques aptes à prévenir l'oblitération cavitaire, quelle que soit la nature, ou la durée, du traitement médical, demeure impressionnante.

Addendum

Since submission of this paper, 2 of the above patients with bronchopleural fistulas have died. One, whose fistula had closed and whose disease had become inactive, died of cor pulmonale. The second had maintained an open fistula and tubercle bacilli in the sputum following resection of the left upper lobe and superior segment of the left lower lobe.

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FIG. 1. M. H., 35-year-old white male. Onset of disease in January, 1943. Right thoracoplasty, 1948; left plombage thoracoplasty in September, 1954. Four different courses of chemotherapy, including at various times streptomycin, PAS, and isoniazid. Resection of apical segment, left upper lobe, on October 21, 1957.

A. Roentgenogram of February 19, 1953, showing left cavities, pneumoperitoneum, and deformity of right thoracoplasty.

B. Roentgenogram of October 18, 1957, just prior to segmental resection. Tubercle bacilli present in sputum. The plombage thoracoplasty is inadequate by later standards. Maximal breathing capacity, 51 liters per minute. Patient required tracheostomy and corticoid therapy postoperatively for pulmonary insufficiency and bronchospasm.

C. Roentgenogram of September 26, 1958; sputum cultures negative for tubercle bacilli. The patient has returned to part-time sedentary work.

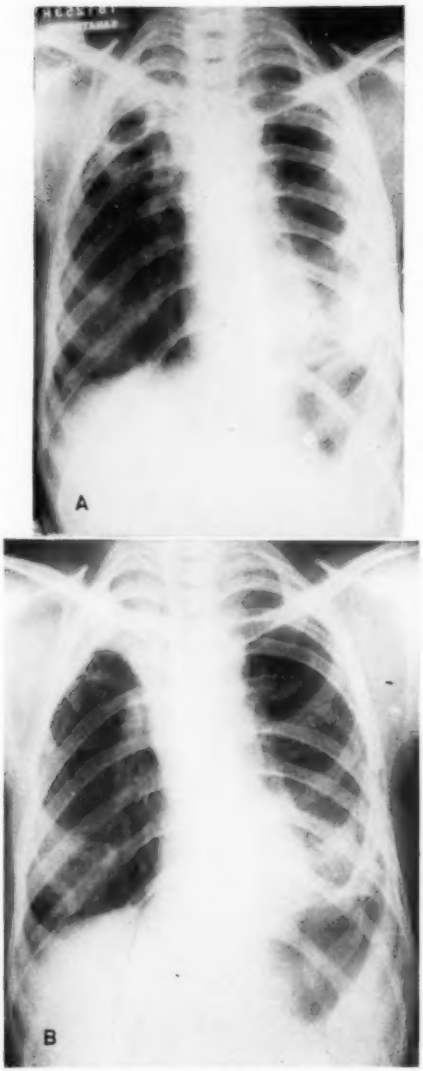

FIG. 2. J. V. J., 32-year-old white female. Onset of disease in September, 1948. Left pneumothorax, January, 1949, to September, 1949, discontinued because of effusion. Monaldi operation on the right in April, 1951. Three different courses of chemotherapy. Maximal breathing capacity of 29 liters per minute increased to 41 liters per minute after left decortication on March 8, 1955, although the patient required tracheostomy and respiratory support for six days postoperatively. On August 23, 1955, the right upper lobe and superior division of the right lower lobe were resected.

A. Roentgenogram of December 18, 1953, showing thickened pleura on the left and cavities on the right.

B. Roentgenogram of September 26, 1956, one year following surgery. One sputum culture positive for tubercle bacilli in August, 1957, but all cultures negative since. Patient shows dyspnea on moderate exertion.

FIG. 3. K. M., a 45-year-old white female. Onset of disease in February, 1955. Admission to hospital in March, 1955. Initial sputum culture showed 4-plus growth in all concentrations of streptomycin and PAS and 4-plus growth in isoniazid concentration of 1 γ of isoniazid although not of 10 γ per ml.

A. Roentgenogram of March 31, 1955.

B. Roentgenogram of September 8, 1955, showing increased disease in the right upper lobe which had previously shown good resolution. Tubercle bacilli were still present in the sputum until viomycin and pyrazinamide were started in December, 1955. The right upper lobe was resected on January 5, 1956.

C. Roentgenogram on September 1, 1958. Sputum cultures are negative for tubercle bacilli and patient is doing full housework.

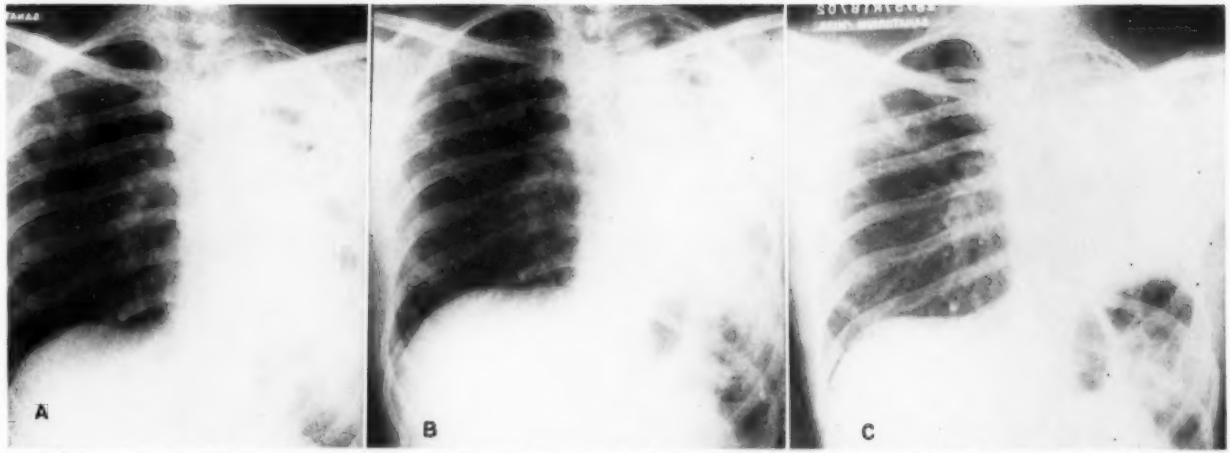

FIG. 4. T.L.B., a 23-year-old Negro female. Onset of disease in March, 1954. Streptomycin, PAS, and isoniazid were started at this time at home. Patient was admitted to the hospital on July 2, 1955, discharging tubercle bacilli resistant to all three drugs employed.

A. Chest roentgenogram of July 7, 1955, on admission.

B. Chest roentgenogram of February 13, 1956, two months prior to left pneumonectomy on April 13, 1956. Note the clearing of the right cavitary lesions.

C. Roentgenogram of January 28, 1957, showing the reactivation of the disease previously noted on the right. All cultures remain negative for tubercle bacilli and there has been some roentgenographic improvement, but laminagrams in September, 1958, still suggest the persistence of cavity on the right.

FIG. 5. D.O.W., a 51-year-old white male. Onset of disease in November, 1951. Pneumoperitoneum in January, 1952, to September, 1954. Streptomycin, isoniazid, and PAS were given from May 15, 1952, to April 4, 1953. It was re-started on March 1,1955. The patient was admitted to the hospital on February 27, 1955, with tubercle bacilli in the sputum. Operations on September 9, 1955, apical segment of left upper lobe; and on January 24, 1956, apical segments of right upper lobe.

A. Chest roentgenogram of November 8, 1951, onset of disease.

B. Roentgenogram of September 6, 1955, just prior to first operation. Sputum still contains tubercle bacilli. Viomycin and pyrazinamide given through period of bilateral resections.

C. Roentgenogram of March 24, 1958. Sputum cultures are negative for tubercle bacilli and patient is doing full-time work.

Case Reports

ENLARGEMENT OF THE INNOMINATE AND SUBCLAVIAN ARTERIES SIMULATING MEDIASTINAL NEOPLASM[1]

ROBERT A. GREEN[2]

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Introduction

Widening of the right superior mediastinum is a frequent abnormal finding in chest roentgenograms, and differential diagnosis is often difficult. Benign and malignant primary neoplasms of the various glandular, lymphatic, and connective tissue structures of the mediastinum are common, as are metastatic tumors, inflammatory and granulomatous processes, cystic lesions, and vascular abnormalities of various types. The roentgenographic findings usually do not clearly point to a single diagnosis, and the symptoms and signs frequently reflect only the contiguity of the lesions to adjacent anatomic structures, and thus are also nonspecific. It is the purpose of this report to discuss enlargement of the innominate artery, a fairly common but infrequently emphasized cause of abnormal densities in this area.

The innominate artery originates at the upper portion of the ascending aorta in the superior mediastinum and courses upward posteriorly and to the right for an average distance of approximately two inches before dividing into the right subclavian and right common carotid arteries. In the postero-anterior roentgenogram of the chest the normal innominate artery lies well within the right border of the superior mediastinum and is therefore not visualized. Its bifurcation is at—or just above—the level of the clavicle, also within the mediastinal density. The normal anatomic relationships of the innominate artery and its appearance on angiocardiograms have been thoroughly described by Honig and Steinberg and their co-workers (1, 2).

When the innominate artery enlarges, it most often projects to the right and thus its margin becomes visible on the chest roentgenogram at the right superior mediastinal border. A number of different pathologic states can cause it to enlarge. Perhaps the most common of these is the buckling or kinking that occurs with hypertension and arteriosclerosis of the aorta. In this condition the artery is elongated, dilated, and tortuous, as well as buckled in its mid-portion, with the convexity usually to the right. Honig and associates (1, 2) believe that hypertension leads to dilatation and arteriosclerosis lengthening of the aorta. The aortic arch becomes elevated, and this in turn leads to tortuosity and kinking of the innominate.

Aneurysms are another major cause of enlargement of the innominate artery and thus account for the abnormal roentgenographic shadow. Aneurysms have been known for many years, and a number of publications have discussed their clinical and roentgenographic characteristics (3–5). In addition, buckling, aneurysms, and pseudo-aneurysms (6) of the subclavian (7) and common carotid (8, 9) arteries can cast relatively similar roentgenographic shadows, although ordinarily involvement of the common carotid artery does not cause an abnormal density on the chest roentgenogram (10, 11).

Recently 4 patients were seen at this hospital who illustrate many of the clinical and roentgenographic aspects of the diagnosis and differential diagnosis of vascular pathology in this area. Three of the 4 patients had significant arterial hypertension; and the fourth, evidence of arteriosclerosis. All were in the seventh decade of life. All had evidence of a right superior mediastinal mass which arose at the level of the aortic arch and extended upwards for a variable distance. In addition, the mass bulged laterally with a smooth convex lateral border in all.

In 3 of the 4 patients the mass caused no symptoms. Horner's syndrome was present in one patient, and another may well have had a related pupillary abnormality. Serologic tests for syphilis were negative in all 4 patients, and no other evidence of syphilis was discovered in any of

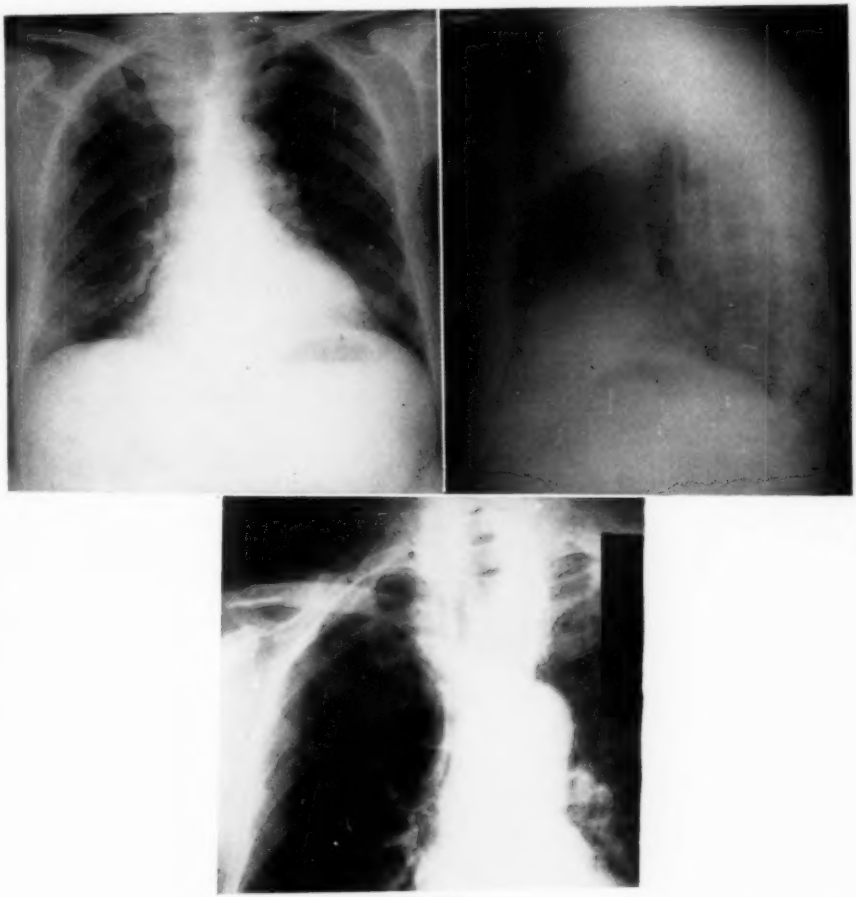
[1] From the Chest Section of the Medical Service, Veterans Administration Hospital, Bronx, New York.

[2] Present address: Veterans Administration Hospital, Ann Arbor, Michigan.

them. In all patients the initial diagnostic impression indicated the possibility of mediastinal tumor. The diagnosis was established by angiocardiography in one case, by thoracotomy in another, and at autopsy in 2 cases. The actual pathology consisted of buckling and tortuosity of the innominate artery in 3 patients, and an aneurysm of the right subclavian artery in the fourth. These 4 cases are further described below.

Case Reports

Case 1. A 67-year-old male was admitted to the Veterans Administration Hospital, Bronx, New York, complaining of recent fatigue. Diabetes mellitus, which required 15 units of protamine zinc insulin daily, had been present for seven years. The patient was a heavy wine drinker. Physical examination revealed moderate obesity and normal vital signs. Blood pressure was 110 systolic, 76 diastolic in mm. of mercury. The right



Case 1

Fig. 1. *A* (Top left). Postero-anterior view of chest. The superior mediastinum is widened to the right. The outer margin is smooth and convex. *B* (Top right). Lateral view of chest. The abnormal density is anterior, and arises at the level of the aortic arch.

Fig. 2 (Bottom). Angiocardiogram, ten seconds after injection. A dilated, elongated innominate artery causes the abnormal shadow.

pupil was slightly larger than the left, but both reacted equally. The fundi were compatible with diabetic retinopathy. Moderate enlargement of the liver and spleen was found. Large ecchymoses were present at injection sites. Laboratory studies revealed thrombocytopenia, elevated blood sugars, and abnormal liver function tests. Bone marrow was normal. A diagnosis of cirrhosis with secondary thrombocytopenia was made.

Roentgenographic studies of the chest revealed a right superior anterior mediastinal mass (figure 1*A* and *B*). The mass arose at the upper border of the aortic arch and curved upward to the clavicle, with a smooth convex outer margin. Pulsations were not demonstrated by fluoroscopy, nor did the mass move with swallowing. One observer believed that coughing caused reduction in the size of the mass. No uptake of radioactive iodine was noted over this region. Angiocardiography demonstrated that the innominate artery was deviated slightly to the right and was tortuous, thus producing the masslike shadow on the roentgenogram (figure 2). The patient was discharged, and when seen one year later was asymptomatic. The roentgenogram was unchanged.

Comment: This patient had no evident arterial hypertension, but diabetes and some evidence of arteriosclerosis were present. An asymptomatic superior mediastinal mass was found which was nonpulsatile but which may have become smaller with increased intrathoracic pressure. The most important diagnostic procedure performed was angiocardiography, and this clearly demonstrated a buckled innominate artery.

The fluoroscopic characteristics of the buckled innominate artery have not been described. When aneurysms were present, pulsations were noted by Gay and Walker (5) in half of their cases, although no changes occurred upon performing the Mueller or Valsalva maneuver. Warfield (3) noticed pulsation in only one of twenty cases of innominate aneurysm. The infrequent demonstration of pulsations is presumably due to the presence of clot within the dilated vessel.

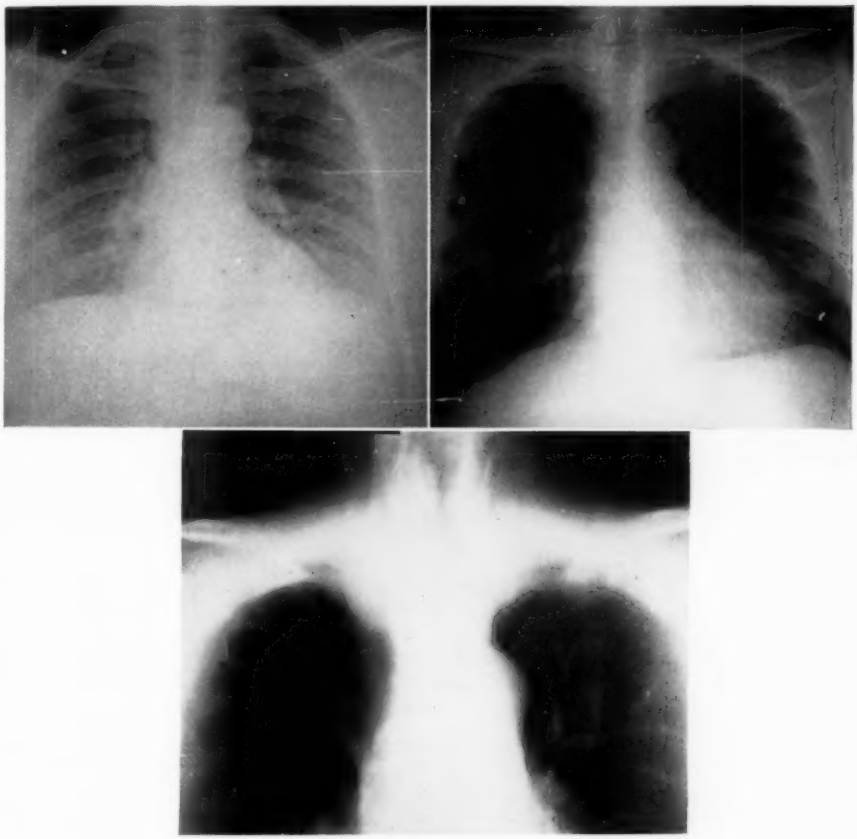
The importance of angiocardiography in the diagnosis of abnormal mediastinal shadows is generally accepted, and has been demonstrated by numerous investigators (12, 13). It was of paramount importance in this case.

Case 2. A 60-year-old male complained of swelling of the right upper eyelid, and a chalazion was found by his ophthalmologist. Following regression of the chalazion, drooping of the eyelid was noted, and the patient was admitted to the Veterans Administration Hospital, Bronx, New York. No history of trauma or visual disturbances was obtained, and systemic review was totally negative. Physical examination revealed a definite right Horner's syndrome, consisting of miosis, ptosis, enophthalmos, and diminished sweating of the right side of the face. The right pupil measured 2 mm., the left 4 mm., in diameter. Although epinephrine in high concentrations dilated both pupils, the right pupil failed to dilate upon application of cocaine. Ophthalmometry confirmed the presence of enophthalmos. Except for blood pressure of 190 systolic, 100 diastolic in mm. of mercury, the remainder of the physical examination was entirely normal. Hematologic and blood chemical studies were normal. Serologic tests for syphilis were negative. An electrocardiogram revealed changes consistent with left ventricular hypertrophy.

Roentgenograms of the chest demonstrated a right paratracheal and apical soft tissue density (figure 3*A*). Its outer margin, consisting of the mediastinal pleural reflection, was smooth. The lesion was more clearly seen on apical lordotic films (figure 3*B*). Roentgenograms of the cervical spine revealed minimal osteoarthritic hypertrophic changes, and an esophagram was normal. The lung fields functioned normally on fluoroscopy, and indirect laryngoscopy showed no abnormalities of the vocal cords. Tomography located the lesion in the anterior mediastinum and demonstrated that a solid density with a smooth convex outer border was present within the mediastinal pleural shadow (figure 3*C*). No abnormal lymph nodes were found by biopsy of the right scalene fat pad.

Because of the Horner's syndrome and abnormal mass shadow, a diagnosis of tumor was made, and exploratory thoracotomy was performed. A redundant, elongated, and tortuous innominate artery was found without aneurysmal dilatation. The vessel made an S-shaped curve with its greater convexity laterally. The entire superior mediastinum and paravertebral area were carefully explored, and no other abnormality was discovered. There was no change in the roentgenogram or Horner's syndrome following the operation or one year later, when the patient was seen at a follow-up visit.

Comment: The abnormal eye findings dominated the clinical picture in this patient, and unfortunately the diagnosis of tumor seemed so definite that angiocardiography was not performed. In retrospect, the roentgenograms suggested the possibility of innominate artery buckling, although the degree of lateral convexity due to buckling was less than in other cases. In fact, it was minimal in the postero-anterior view and only clearly demonstrated in the lordotic film. Actually, this diagnosis can never be made with certainty on conventional roentgenograms (1); however, the anterior location of the shadow, its smooth curved outer margin, and its origin



Case 2

FIG. 3 *A* (Top left). Postero-anterior view of chest. The right superior mediastinum is wide. *B* (Top right). Apical lordotic view of chest. Note the convex outer border. *C* (Bottom). Tomogram, 12 cm. from posterior chest wall. The density is anterior and the border smooth.

from the region of the aortic arch are all suggestive. Hypertension was present, but no stigmata of arteriosclerosis were described.

The eye findings in this patient are of great interest, and apparently are by no means unique when vascular abnormality is present in this area. Pupillary abnormalities, not otherwise described, were found in 44 per cent of Gay and Walker's series (5) of innominate aneurysms; it would appear that the pupil on the side of the aneurysm was dilated in most of the patients, due to pressure upon and stimulation of the cervical sympathetic nerves by the aneurysm. Similar findings are reported by Warfield (3). It is of interest to note that the right pupil was described as larger than the left in *Case 1*, although further studies were not performed to elucidate the abnormality. In *Case 2*, Horner's syndrome was demonstrated both clinically and by the pupillary reactions to cocaine and epinephrine (14); it might be inferred that the pressure from the abnormal vessel had eventuated in paralysis of the sympathetic nerves. Honig and associates (1) mention a patient with Horner's syndrome and a syphilitic aneurysm; the patient of the present case would appear to be the first described in whom this occurred due to a non-aneurysmal buckled artery.

Case 3. A 64-year-old male was transferred to the Veterans Administration Hospital, Bronx, New York, for study of a possible mediastinal tumor. He had been hospitalized elsewhere because of mental confusion, dyspnea, and hypertension of unknown duration. There was a long history of chronic renal disease. Cachexia, confusion, and marked arteriosclerosis of the peripheral arteries were noted. Blood pressure was 220 systolic, 120 diastolic in mm. of mercury. Serologic tests for syphilis were negative. Azotemia and albuminuria were present, and renal function tests showed marked impairment. Roentgenographic studies of the kidneys revealed an atrophic left kidney and a possible left suprarenal mass. A large superior mediastinal density was present on the chest film (figure 4*A*). Bronchoscopy and scalene node biopsy were unrevealing, and the patient was transferred to this hospital for further investigation.

Here, hypertension and evidence of renal insufficiency persisted, and mild diabetes was also discovered. Additional roentgenograms revealed extrinsic pressure on the esophagus at the site of the mass which was thought to have increased in size (figure 4*B*). The aorta was uncoiled. The cerebral symptoms were believed to be due to cerebral arteriosclerosis, but metastatic neoplasm could not be ruled out. One observer at fluoroscopy noted pulsations in the mass which were not thought to be transmitted, and a diagnosis of innominate arterial aneurysm was suggested. Angiocardiography was unsuccessful. Renal failure progressed, bronchopneumonia developed, and the patient died.

At autopsy an atrophic left kidney and bilateral pyelonephritis were found. Multiple areas of old encephalomalacia were present. Marked atherosclerosis and calcification of the aorta and its tributaries were noted. The innominate artery was wide and a large aneurysm measuring 5 to 6 cm. in diameter was found at and distal to its bifurcation (figure 5). The aneurysm actually involved the subclavian artery rather than the innominate. It was filled with laminated thrombus, but the lumen was patent. Microscopic sections did not reveal evidence of syphilis.

Comment: Marked hypertension and arteriosclerosis were both present in this patient, as well as diabetes and renal insufficiency. Although innominate arterial aneurysm was suspected clinically, the mediastinal and possible suprarenal masses in addition to the progressive cerebral dysfunction suggested the possibility of tumor. This seemed more likely when the mass appeared to increase in size. As autopsy revealed an aneurysm of the subclavian artery, this apparent enlargement may have represented an increase in the size of the aneurysm or may have been due to a difference in roentgenographic technique. The degree of inspiration at the time of exposure of the film in figure 4*B* is considerably less than that in figure 4*A*. This would tend to elevate all of the thoracic contents, including the aorta, and thus further buckle the innominate artery to the

Case 3

FIG. 4 *A* (Left). Postero-anterior view of chest. The heart is enlarged, the aorta prominent, and a large density is present in the right superior mediastinum. *B* (Right). Four months later. The abnormal shadow appears larger.

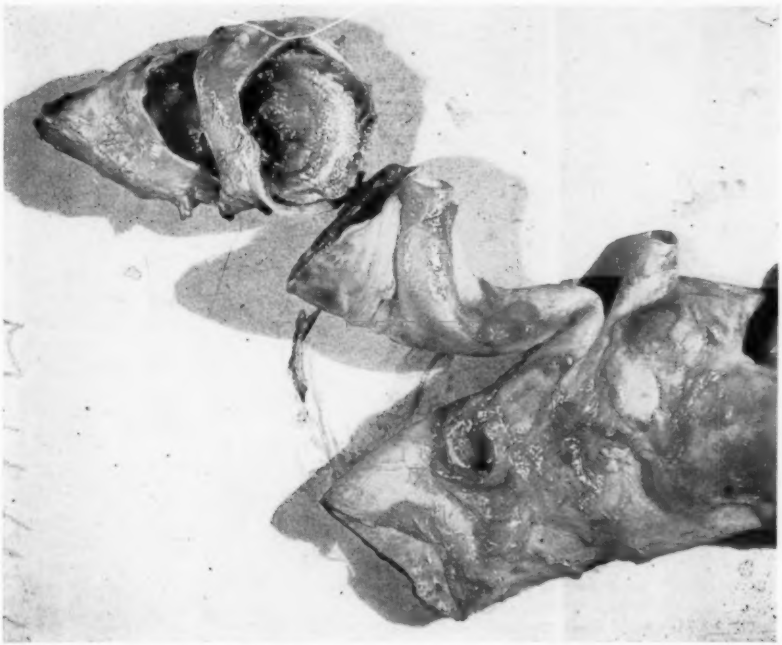


FIG. 5. *Case 3.* Autopsy specimen. The innominate artery is wide. At its bifurcation a large aneurysm of the subclavian arises.

right, simulating a real enlargement of the density. In future patients, an attempt should be made at fluoroscopy, or by films taken in inspiration and in expiration, to determine if abnormal vascular shadows buckle to the right significantly more than do solid tumors. If so, this might become a valuable sign in differential diagnosis. It should also be noted that fluoroscopic pulsations were seen in this patient in spite of the presence of a large amount of laminated thrombus, but widening upon expiration was neither looked for nor described.

The thoracic aorta was distinctly uncoiled and tortuous in the patient of *Case 3.* In Warfield's (3) series of twenty cases of innominate arterial aneurysm, involvement of the aorta was present in all cases and, in fact, Warfield believed that innominate arterial aneurysms probably did not occur without prior or concomitant involvement of the aorta. Gay and Walker (5), however, found a normal aorta in one-third of their patients. Both writers referred basically to syphilitic aortitis and syphilitic innominate aneurysm. Serologic tests were positive in 75 per cent of the patients in both groups, and Gay and Walker (5) believed that syphilis was the cause of the aneurysm of the innominate artery in all of their eighteen cases, expressing the opinion that an authenticated case of a purely arteriosclerotic innominate aneurysm was not to be found in the literature. The serologic test was negative in *Case 3,* and the microscopic sections of the major vessels did not reveal any evidence of syphilitic involvement.

After *Cases 1* to *3* were seen and the present writer and his associates had become familiar with the type of shadow that enlarged brachiocephalic vessels in this area cast upon the roentgenogram, as well as the clinical background of hypertension and arteriosclerosis in most patients, many instances of various degrees of this abnormality were diagnosed during the routine study of patients admitted for other reasons. In most of these patients no further diagnostic work was done, as the lesions were asymptomatic and appeared typical from a clinical and roentgenographic standpoint. Occasionally, however, the

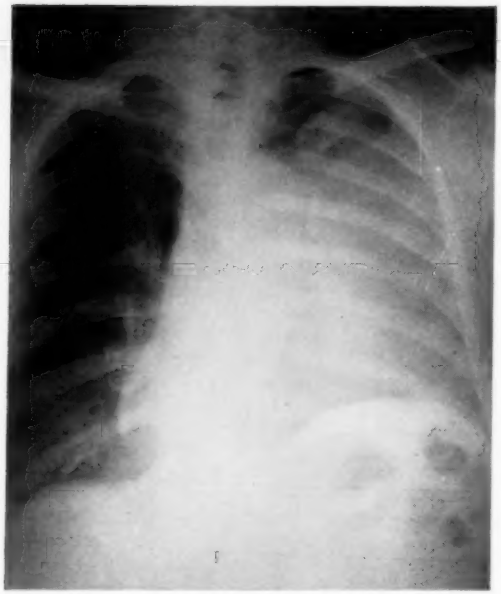


FIG. 6. *Case 4.* The left upper lobe is nonaerated and the left diaphragm is elevated. Note the curved density extending up and to the right from the aortic arch.

opportunity to confirm the diagnosis arose. A report on one such patient is presented below.

Case 4. A 62-year-old male was admitted to the hospital complaining of cough, weight loss, and hoarseness. Breath sounds were diminished over the left anterior chest. Blood pressure was 170 systolic, 110 diastolic in mm. of mercury. A roentgenogram revealed opacification of the entire left upper lobe and a smooth curvilinear shadow in the right superior mediastinum (figure 6). At bronchoscopy a friable bleeding mass at the orifice to the left upper lobe was visualized. The left vocal cord and left diaphragmatic leaflet were paralyzed, and the situation was deemed inoperable.

The superior mediastinal shadow was suggestive of a buckled innominate artery but, in view of the presence of a carcinoma, metastatic disease in the area was thought likely. The patient gradually failed, death ultimately being due to pericardial involvement by the tumor. At autopsy a bronchogenic carcinoma was found at the left lung root which had invaded the surrounding structures, including the pericardium and myocardium. The great vessels were atheromatous, and the innominate artery tortuous. No tumor was present in the superior mediastinum.

DISCUSSION

The clinical picture of aneurysms of the innominate artery has been described by many writers (1, 3, 5, 8). A pulsatile mass at the base of the neck, local pain, cough, dyspnea, hoarseness, dysphagia, pupillary abnormalities, and pain, temperature changes, and clubbing of the fingers in the right arm appear to be frequent. The symptoms are progressive, and death may result from eventual rupture of the aneurysm. Because of this a surgical approach has been advocated by various writers (4, 9, 15, 16).

Buckling, on the other hand, is a benign condition with no surgical implications, of importance mainly because of the difficulty in differentiating it from other more serious abnormal conditions in the superior mediastinum. In fact, one of the patients of Honig and associates (2), as well as the patient of *Case 2* of this series, was subjected to thoracotomy before the correct diagnosis was made. Symptoms are rarely ascribed to the buckling in most discussions of the subject (1, 2, 8, 10), and the only physical findings noted are increased pulsations, occasionally with a bruit, at the base of the neck. It has been suggested that, when the pulsatile swelling is noted, the kinking involves mainly the common carotid in addition to—or rather than—the innominate (11). This is especially true if an abnormal roent-

genographic shadow does not accompany the pulsation (10). Horner's syndrome, which was present in *Case 2*, is a rather unique finding, as previously discussed. Unlike aneurysms, which are infrequently encountered, buckling or elongation and tortuosity of the innominate without buckling is quite common: Honig and co-workers found an incidence of 17 per cent in an angiocardiographic study of hypertensive and/or arteriosclerotic patients (2). The majority of instances of buckling are found in this type of patient, and this was true of the 3 cases in the present series. In each of these 3 patients, roentgenographic evidence of widening of the thoracic aorta of varying degree, indicating arteriosclerotic change, was present. This was also found in the series of Parkinson and associates (10).

The differential diagnosis of right superior mediastinal shadows includes substernal thyroid, benign and malignant tumors, congenital cysts, granulomas, aneurysm of the aortic arch, dilatation of the superior vena cava, nonspecific mediastinal pleural thickening, and opacification of the paramediastinal portion of the right upper lobe by various processes. When the shadow is smooth in contour, arises at the level of the aortic arch, has a convex outer border, and occurs in a hypertensive or arteriosclerotic patient with few or no symptoms ascribable to the shadow, a diagnosis of enlargement of the innominate artery or its tributaries should be seriously considered.

Summary

A report on 4 patients has been presented in whom enlarged innominate arterial shadows simulated mediastinal neoplasms. The actual pathology consisted of buckling of the innominate artery in 3 patients and an arteriosclerotic aneurysm of the subclavian artery in the fourth. Aspects of the differential diagnosis of these conditions have been discussed. Enlargement of the innominate artery should be considered when hypertensive or arteriosclerotic patients are noted to have asymptomatic right superior mediastinal shadows of the type described.

Sumario

Hipertrofia del Tronco Braquiocefálico de la Arteria Subclavia Simulando Neoplasia del Mediastino

Se presentan los casos de 4 enfermos en quienes las sombras del tronco braquiocefálico hipertrofiado simulaban neoplasias mediastínicas. La verdadera histopatología consistía en acodadura del tronco braquiocefálico en 3 enfermos y en aneurisma arteriosclerótico de la arteria subclavia en el otro. Se discuten los aspectos que reviste el diagnóstico diferencial de dichas dolencias. Hay que considerar la hipertrofia del tronco braquiocefálico cuando se nota que enfermos hipertensos o arterioscleróticos muestran imágenes asintomáticas del género descrito en la porción superior derecha del mediastino.

Resume

Elargissement des artères innominées et sous-clavières simulant une néoplasie médiastinale

Quatre patients chez lesquels l'opacité d'une artère innominée élargie simulait une néoplasie médiastinale ont été présentés. Les lésions pathologiques actuelles consistaient en une distension de l'artère innominée chez 3 malades et un anévrisme artérioscléreux de l'artère sous-clavière chez l'autre. Les aspects du diagnostic différentiel dans ces conditions ont été commentés. Il y a lieu de penser à l'élargissement de l'artère innominée, quand on observe chez des malades hypertendus ou artérioscléreu de telles opacités médiastinales asymptomatiques dans la région supérieure droite du thorax.

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TOXIC PSYCHOSIS DUE TO ISONIAZID[1]

Report of Three Cases with Studies of Patterns of Isoniazid Inactivation in the Serum

AVRUM B. ORGANICK

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Introduction

Toxic psychosis is an uncommon complication of isoniazid treatment for tuberculosis; nevertheless, since 1952, when isoniazid was first introduced for general use, reports of toxic psychosis have appeared with some regularity in the literature. Such reports usually have fallen into one or the other of two groups. The first is the group of reports of toxic psychosis which occurred in patients who received isoniazid in doses of 4 to 10 mg. per kg. in the treatment of tuberculosis (1–14); the second is the group of reports of cerebral toxicity in persons who ingested massive amounts of isoniazid, usually with suicidal intent (15–18).

Between May, 1957, and May, 1958, on the 36-bed tuberculosis ward at the Denver General Hospital, toxic psychosis was seen in 3 patients who received a dosage of 12 mg. per kg. per day. For three reasons it is timely to report these cases: (*1*) to call attention again to psychosis as a manifestation of isoniazid toxicity at a time when the use of isoniazid in higher doses (12 mg. per kg. per day) is gaining wider acceptance (19); (*2*) to report the results of determinations of patterns of inactivation of isoniazid in the serum of these patients; and (*3*) to describe how patients manifesting toxic organic reactions on a 12 mg. per kg. per day dose may tolerate isoniazid in lower doses.

Case Reports

Case 1. (D.G.H. #78628). T.W., a 43-year-old Negro waiter, previously in good health, was admitted to the Denver General Hospital on September 10, 1957, with hemoptysis. A chest roentgenogram revealed a cavity at the level of the first interspace on the right with infiltration in the right lower and left mid-lung field. The sputum was positive on microscopy for acid-fast bacilli, and subsequently cultures of the sputum proved positive for *M. tuberculosis*. Test doses of isoniazid were given, and blood was drawn for determination of serum concentrations by the method described by Bell and Riemensnider (20); when these determinations were completed, treatment was started on September 20, 1957, consisting of isoniazid, 900 mg. per day (12 mg. per kg. per day); streptomycin, 1.0 gm. per day; PAS, 12.0 gm. daily; and pyridoxine, 50 mg. per day. Para-aminosalicylic acid was discontinued on October 10 because of the appearance of febrile episodes representing an idiosyncrasy to PAS. On October 21 the patient complained of loss of hearing. On examination he was found to be lethargic, slow of speech, and confused. He was ataxic and walked on a wide base. The ataxia and loss of hearing were believed to be manifestations of streptomycin toxicity and, accordingly, streptomycin was discontinued on October 21. Over the next several days the patient's ataxia diminished, but he became increasingly lethargic and slow of speech. On October 29 his confusion was more marked, the patient wandered aimlessly about the ward, and on October 30 during the early morning hours he was found crouching beneath the bed, fearful, and shouting that his bed was on fire. Nevertheless, he recognized the nurse and cried "What am I doing here?" It was clear that the patient's behavior represented a toxic delirium, probably due to isoniazid; accordingly, isoniazid, as well as all other medication, was discontinued on October 30. The patient was fearful and refused a lumbar puncture at this time but consented to it on November 5. The lumbar puncture revealed normal pressure, cells, and values for protein, sugar, and chlorides. Clearing of the sensorium over the next fourteen days was gradual but steady following cessation of isoniazid.

Repeating a test dose of PAS on November 29, a febrile reaction was noted almost immediately, and all further efforts to administer PAS were abandoned. Streptomycin was tolerated in a test dose of 0.5 gm., and regular daily 0.5-gm. doses of streptomycin were resumed on December 5.

Further thought was then given to the use of isoniazid again, this time in lower doses, in order to supplement streptomycin which, at that time, was the only antituberculous agent the patient was receiving. Serum bioassay after a single test dose of 4 mg. per kg. of isoniazid had revealed serum isoniazid concentrations of 3.2 γ per ml. at two hours, and 1.6 γ per ml. at six hours. These data indicated that the patient belonged to the group of patients who are capable of achieving high serum concentrations of biologically active

[1] From the Tuberculosis Service, Denver General Hospital; and the Department of Medicine, University of Colorado School of Medicine, Denver, Colorado.

isoniazid and who inactivate isoniazid slowly. It was believed at the time that high and sustained serum concentrations of isoniazid played a role in the production of the toxic psychosis. It was hoped that serum concentrations might be obtained with a smaller dose of isoniazid which, although still within the therapeutic range, might be better tolerated. After obtaining blood for determination of serum-isoniazid concentration following a test dose of 2 mg. per kg., isoniazid was readministered in a dose of 150 mg. per day (2 mg. per kg. per day) starting December 19. This dose was well tolerated without return of confusion, memory loss, or delirium.

Serum bioassay following the 2 mg. per kg. test dose revealed concentrations of 1.6 γ per ml. at two hours and of 0.8 γ per ml. at six hours. Following a test dose of 50 mg. or 0.6 mg. per kg. (representing one-third of the total daily dose), serum isoniazid concentrations were 0.8 γ per ml. at two hours and 0.4 γ per ml. at six hours.

Streptomycin was reduced in dosage to 0.5 gm. twice a week because of pruritus, and eventually it was discontinued on March 21, 1958, because of rising blood urea nitrogen (to 45 mg. per 100 ml.). Urine examined on admission revealed microscopic pyuria and hematuria. Investigation of the kidneys revealed a nonvisualized kidney on the left and caliectasis on the right. Urine cultures for *M. tuberculosis* were never positive, but the presence of renal tuberculosis was suspected.

Chest roentgenograms revealed marked improvement, and serial laminagrams demonstrated closure of the cavity present on admission. The sputum became negative for tubercle bacilli after October, 1957, and has remained so. The patient was discharged with arrested disease on August 1, 1958.

Comment: This patient at first manifested the rather complicated clinical picture of two types of toxicity combined, that of eighth-nerve toxicity due to streptomycin and of a toxic confusional state due to isoniazid. Renal insufficiency favored the development of eighth-nerve toxicity. Did renal insufficiency in any way favor the development of toxic psychosis following isoniazid? Renal insufficiency has not been described as a contributing factor in the reported cases of isoniazid psychosis. Mitchell and Bell (21) found no correlation between renal insufficiency and concentrations of biologically active isoniazid in the serum.

This patient however was a "slow inactivator" of isoniazid, and presumably had high serum concentrations of isoniazid (although these were not measured) during the time when he was showing signs of mental confusion. It is of interest, therefore, to review a previous case of toxic psychosis due to isoniazid in which serum inactivation studies had been done.

Case 2 (D.G.H. # 67830). H.C., a 65-year-old practical nurse, entered the Denver General Hospital on March 21, 1957, with a reactivation of pulmonary tuberculosis. Blood studies revealed a hypochromic, microcytic anemia with a hemoglobin of 8.5 gm. per 100 ml. On May 15 two sputum smears were positive for acid-fast bacilli and subsequently cultures of these specimens proved positive for *M. tuberculosis*. After testing of serum isoniazid concentrations following a standard 4 mg. per kg. test dose, treatment was started on May 24, 1957, with isoniazid, 700 mg. per day (12 mg. per kg.); PAS, 12 gm. daily; streptomycin, 0.5 gm. daily; and pyridoxine, 50 mg. per day. On May 27, three days after starting treatment, the patient complained that she was mentally unclear, that her "head was whirling," and that she feared she was losing her mind. She was distressed over finding herself doing something and not knowing why, finding herself walking somewhere and not knowing why she had set out. Because of these symptoms and the possibility that they represented a toxic psychosis, isoniazid was discontinued on May 27. On the following day the patient was much less anxious, had no further complaints of memory loss or confusion, and thereafter she remained mentally clear.

On July 1, however, isoniazid was restored in a dosage of 300 mg. per day (6 mg. per kg. per day). On July 7 the patient again complained that the isoniazid was "affecting her mentally." Over the next several days she reported that, while she felt well in the mornings, towards evening she would have periods of "blanking out of the mind" and that she would feel "off the beam."

On July 18 the patient's case was reviewed, and consideration was given to the serum-isoniazid concentrations. After a test dose of 4 mg. per kg. of isoniazid, the serum concentrations were 0.4 γ per ml. at two hours and less than 0.2 γ per ml. at six hours. These data indicated that the patient was a "rapid inactivator" of isoniazid. Accordingly, the dose of isoniazid was increased to 800 mg. per day (16 mg. per kg. per day). Following this increase in dosage, the patient's complaints about and anxiety over her memory loss increased. On July 30 she complained that she felt "just muddled up all the time," and on August 3 that she could "hardly remember her own name." On August 6 she feared she would be unable to control her impulses to jump out of the window and she refused thereafter to take any more isoniazid. Isoniazid was therefore discontinued and her mental status returned to normal within two days.

Comment: Administration of isoniazid on two separate occasions was followed by the development of symptoms of confusion, memory loss, and anxiety, the symptoms being more severe with increase in the dose of isoniazid. Each time the symptoms disappeared promptly when isoniazid was discontinued.

The administration of isoniazid, therefore, was

a necessary factor in the development of this picture. In this case an additional factor seems to have been operative, that of anemia. Lewis (22) in 1952 reported that anemia was a factor in predisposing to the neurologic side effects of isoniazid. Nervousness, irritability, and somnolence were more frequently seen in older persons and in patients with anemia. After careful study of this patient, it was concluded that the anemia was related to chronic infection. The anemia later disappeared as her general condition improved with treatment of her tuberculosis with streptomycin and PAS.

Serum inactivation studies revealed that this patient was a "rapid inactivator." High serum concentrations of free, biologically active isoniazid could not be presumed to have played a role, although serum concentrations were not measured during the time she was manifesting symptoms of the toxic state.

During the time that these 2 cases were being reviewed, a third case came to the attention of the writer. This case represented a somewhat different situation, namely, an exacerbation due to isoniazid of a toxic delirium in a person who had recently had one.

Case 3 (D.G.H. #89344). R.B., a 76-year-old Russian Jewish widow, was transferred to the Denver General Hospital on March 14, 1958. A diagnosis of active, far advanced pulmonary tuberculosis had been made on the basis of a chest roentgenogram and on the finding of acid-fast bacilli on microscopy of the sputum.

Two days prior to being transferred to the Denver General Hospital the patient began to express paranoid ideas and attempted suicide by swallowing several sharp metallic objects and coins. On admission to the Denver General Hospital she was found to be a thin, elderly, uncooperative, resistive woman who expressed ideas of futility, hopelessness, and paranoia, stating: "What's the use? I am going to die anyway. Why don't you just kill me and get it over with? They killed my brother and now they are going to kill me because I know too much." On physical examination, dullness, bronchial breath sounds, and rales were noted over the left upper lungfield. A chest roentgenogram revealed far advanced pulmonary tuberculosis with a 4-cm. cavity in the left upper lobe; acid-fast bacilli were found in the sputum, and the cultures were positive for *M. tuberculosis*.

During the first several days following admission the patient's mental status improved steadily; she became more cooperative, expressing fewer feelings of depression and ceasing to express paranoid ideas. After ten days it was believed that she had returned essentially to normal. It was the opinion of the psychiatric consultant that the patient had a personality with paranoid features and that the acute reaction represented a toxic delirium, the patient having received meperidine (Demerol®), chloral hydrate, and an antihistaminic preparation called Triaminic® while at another hospital.

Following determination of serum-isoniazid concentrations, specific antituberculous treatment was started on March 24, 1958, as follows: isoniazid, 12 mg. per kg. per day or 500 mg. per day in divided doses; streptomycin, 0.5 gm. daily; PAS, 12.0 gm. per day; and pyridoxine, 50 mg. per day.

Because of pruritus, streptomycin and PAS were discontinued on May 2.

During the sixth week of isoniazid therapy, the patient began to report hearing voices of other patients and of the ward personnel whispering, "She is going to die tonight; she is going to die tonight." When the doctors were present in the room, however, she heard no voices. She showed some awareness of her difficulty stating that she was "sick in the head" and that her "mind isn't working right." Over the next several weeks the patient reported that she heard the voices all day long, and she became increasingly anxious. These "voices" were misinterpretations of actual voices of nurses in the nurses' station or of the hospital page-system loudspeaker; they probably represented illusions rather than hallucinations. It was believed that these phenomena represented a toxic-organic psychosis, probably due to isoniazid; accordingly, isoniazid was discontinued on June 6.

Over the next several days the patient became less anxious and fearful, and she reported that the "voices" had disappeared entirely by the tenth day and that she felt calm and hopeful.

On June 16 she received a single test dose of isoniazid of 650 mg. or approximately 15 mg. per kg. Eight hours after this single dose she again appeared to be fearful and anxious, and twenty-four hours later she again expressed overt ideas of persecution, stating: "They are going to kill me tonight in this bed like a rat. I cannot stay here any longer." Extremely fearful, she dressed and refused to return to her room, whereupon she was transferred to the psychiatric ward. The "voices" disappeared and she was much less agitated by June 19, but she persisted in her conviction that the tuberculosis ward was like a "Nazi concentration camp."

On July 1, 1958, the patient was transferred back to the tuberculosis ward, accepting this calmly. Over the next seven days, still not receiving isoniazid, she was observed to be calm without fear or anxiety, and she denied hearing any voices. At this time the patient was receiving no chemotherapy for her tuberculosis. Since isoniazid was still required for treatment of the disease, it was important to determine if she would tolerate isoniazid in daily doses of less than 12 mg. per kg. Accordingly, on July 8 isoniazid was resumed in a total dose of 5 mg. per kg. per day in

three divided doses. After two weeks on this dose she again began to manifest mild anxiety and would admit on questioning to some ideas of persecution. She has been maintained on this dose of isoniazid for three months without any increase in symptoms, and she remains cooperative. Unless mental deterioration occurs, no further reduction in the dose of isoniazid is contemplated.

Serum bioassay to determine the pattern of isoniazid inactivation revealed that this patient after a single test dose of 4 mg. per kg. achieved isoniazid concentrations in the serum of 0.4 γ per ml. at two hours and less than 0.2 γ per ml. at six hours, indicating that she was a "rapid inactivator."

Comment: The temporal relationship between the administration of isoniazid and the appearance of anxiety and paranoid ideation observed repeatedly in this patient leaves little doubt that administered isoniazid was the cause of the toxic state each time. This causal relationship seemed clear despite the underlying paranoid personality disorder in the patient and the fact that drugs other than isoniazid had produced similar reactions in the past. Wolff and Curran (23) describe the "dysergastic reaction" or toxic delirium and point out that the content of such reactions is dependent upon the underlying personality disorder rather than upon the offending agent. Paranoid misinterpretations in the toxic state, they point out, are common in persons who have been inordinately suspicious. These writers describe a case in which a toxic state appeared on two occasions with different offending agents each time and in which the toxic states were similar.

Discussion

The 3 patients described in this report developed classic features of toxic or confusional psychosis while receiving isoniazid in a dosage of 12 mg. per kg. per day. In all of these cases it was possible to demonstrate that, while definite symptoms appeared at the 12-mg. per kg. dosage of isoniazid, reduction in dosage resulted in either the complete disappearance (as in *Case 1*), or in the diminution of the severity, of the symptoms (as in *Cases 2* and *3*). These cases would indicate that confusional psychosis due to isoniazid represents a dose-response phenomenon and not drug idiosyncrasy. Abundant evidence supports this view. Numerous reports indicate that toxic confusional states appear quite regularly in persons who ingest, usually with suicidal intent, massive amounts of isoniazid. In contrast, toxic psychosis is extremely uncommon in patients receiving isoniazid in doses of 3 to 5 mg. per kg. per day. In individual cases, Hunter (1) and Pleasure (9) demonstrated that symptoms would appear on the higher of two doses of the drug, but that lower doses would be well tolerated.

With the results of the serum isoniazid determinations (20, 21) in the patients whose cases are reported here, some correlation was sought between the pattern of inactivation of isoniazid and the appearance of toxic psychosis. A priori, it would be expected that toxic psychosis would be associated with a pattern of slow inactivation of isoniazid and, by inference, perhaps high concentrations of biologically active, or free, unconjugated isoniazid. Examination of the data from *Case 1* is in accord with this hypothesis. The second and third cases, however, failed to show any correlation between slowness of inactivation of isoniazid and cerebral symptoms. The patients of the second and third cases fall into the category of rapid inactivators of isoniazid; they did not achieve high serum concentrations at two hours and they did not maintain even small concentrations of isoniazid at six hours.

Some comment on the relationship of peripheral neuritis and toxic psychosis as manifestations of isoniazid toxicity seems in order. In the first cases of confusional psychosis due to isoniazid to be reported, Hunter (1) and McConnell and Cheetham (2) described the concomitant appearance of peripheral neuritis in the same patient. It seems, however, that cases of psychosis and neuritis occurring together in the same patient represent ingested isoniazid producing two different types of toxicity. Certainly, peripheral neuritis was a much more common type of toxic manifestation before the general use of pyridoxine, and it occurred most of the time without psychosis. Furthermore, toxic psychosis unaccompanied by peripheral neuritis has been reported numerous times. In the cases described in this report, all 3 patients received pyridoxine in doses of 50 mg. per day while receiving isoniazid in a dose of 12 mg. per kg. While pyridoxine may have prevented the emergence of peripheral neuritis (24), it did not protect these patients against the development of toxic confusional psychosis.

Ross (25), reporting recently on the use of pyridoxine hydrochloride to prevent isoniazid

toxicity, found the same thing, namely, that pyridoxine did not prevent the development of toxic psychosis in patients who received 20 mg. of isoniazid per kg. per day. Biehl (26) had also made the same observation with three of forty-eight patients who developed toxic psychosis on 20 mg. of isoniazid per kg. per day despite having received pyridoxine.

Summary

Three cases of toxic psychosis which developed in patients receiving isoniazid in doses of 12 mg. per kg. per day are reported. It is timely to call attention to this complication of isoniazid therapy when higher doses of isoniazid are being employed in the treatment of tuberculosis.

Studies of rates of inactivation of biologically active isoniazid in the serum were obtained in these 3 patients. One patient was a slow inactivator of isoniazid; the other 2 were rapid inactivators. It was not possible to make any correlation between slow inactivation of isoniazid and toxic psychosis. Administration of 50 mg. per day of pyridoxine did not prevent toxic psychosis. Two of the 3 patients who developed a toxic psychosis on a 12 mg. per kg. daily dose of isoniazid tolerated lower doses.

Sumario

Psicosis Tóxica Debida a la Isoniacida: Presentación de Tres Casos con Estudios de los Patrones de la Inactivación de la Isoniacida en el Suero

Preséntanse 3 casos de psicosis tóxica observada en enfermos que recibieron isoniacida a dosis de 12 mg. por kg. al día. Parece oportuno llamar la atención sobre esta complicación de la isoniacidoterapia hoy día cuando se emplean dosis más altas de la droga en el tratamiento de la tuberculosis.

Se hicieron estudios de los coeficientes de inactivación de la isoniacida biológicamente activa en el suero en 3 enfermos. Un paciente era un inactivador lento de la isoniacida; los otros 2 eran inactivadores rápidos. No fué posible descubrir la menor correlación entre la inactivación de la isoniacida y la psicosis tóxica. La administración de 50 mg. diarios de piridoxina no impidió la psicosis tóxica. Dos de los 3 enfermos que manifestaron una psicosis tóxica con una dosis diaria de 12 mg. de isoniacida por kg. de peso toleraron dosis más bajas.

Resume

Psychose toxique due à l'isoniazide: Rapports sur trois cas et étude des modalités de l'inactivation de l'isoniazide dans le sérum

L'auteur rapporte trois cas de psychose toxique survenant chez des malades recevant l'isoniazide à la dose de 12 mg/kg par jour. Il est opportun d'attirer l'attention sur cette complication du traitement par l'isoniazide lors de l'accroissement des doses d'isoniazide dans le traitement de la tuberculose.

L'étude des taux d'inactivation de l'isoniazide sérique biologiquement active a été effectuée chez trois malades. Un malade inactivait lentement l'isoniazide, les deux autres l'inactivaient rapidement. Il n'a pas été possible d'établir une corrélation quelconque entre l'inactivation lente de l'isoniazide et la psychose toxique. L'administration de 50 mg de pyridoxine par jour n'a pas prévenu l'apparition de la psychose toxique. Deux des trois malades ayant présenté une psychose toxique quand ils recevaient une dose de 12 mg/kg par jour toléraient des doses plus faibles.

Acknowledgments

The writer wishes to thank Dr. David Kazzaz, Psychiatric Division, Denver General Hospital, who saw all 3 patients mentioned in this report in consultation and who offered encouragement in the preparation of this paper; and Dr. Roger S. Mitchell and Mr. D. K. Riemensnider from the Colorado Foundation for Research in Tuberculosis, who made possible the determinations of the serum isoniazid concentrations.

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Notes

EVOLUTION OF MURINE LEPROSY

Studies on the evolution of murine leprosy in rats and mice have been reported by several investigators,[1-6] based on histologic changes or comparative bacillary counts. In what seems to be the first quantitative study, Hobby and associates[7] have enumerated the bacilli in a serial study of lesions in the spleen.

The subject of this report is the progress of the disease in mice, infected intraperitoneally with the Hawaiian strain of *M. leprae murium*. Four measurements are used: (*1*) number of acid-fast bacilli in the macrophages of the peritoneal exudate; (*2*) number of infected cells in the mesentery; (*3*) estimated bacillary population of the spleen, and (*4*) estimated bacillary population of the liver.

Female, albino mice of the general purpose strain, N.I.H., weighing 16 to 22 gm. were inoculated with 0.5 ml. of a 1:30 suspension made from omenta and pelvic fatty pads of mice infected four to five months previously. Suspensions of autoclaved lesions were used as a control.

Animals were sacrificed at one, three, five, seven, eleven, and fourteen days, and thereafter at weekly intervals for ten to thirteen weeks. On each occasion, 5 animals were used for peritoneal impression smears. Groups of 10 were used for all other purposes except for counts of living bacilli made at the end of three months, for which 20 animals were employed for each suspension.

Impression smears were made on glass slides by light touch on the freshly exposed and undisturbed abdominal viscera. For fixation, the slides were placed immediately in Helly's fluid. The percentages of various types of cells and the proportion showing phagocytosis were recorded. The number of bacilli in macrophages was estimated according to the method described below.

Mesenteric spreads were prepared by placing on glass slides a loop of the lower portion of small intestine, including three to five large sections of the mesentery. Spreads were made about fifteen minutes after killing, blood in the loop was drained off and, after drying, the intestine and blood vessels were cut off. Spreads were fixed in Helly's fluid. The number of infected cells in the mesentery was graded according to the scoring method described below.

The technique of microbial enumeration used by Hobby and associates[7] was employed with a few modifications. Suspensions of spleen and liver were made from the pooled organs of each group. The tissues were homogenized in a Waring Blendor, using a semimicro Monel metal container (Cenco #17246B). Four milliliters of ice water were used for each spleen and 7 ml. for each liver, minus the estimated volume of the organ, taking grams of weight as milliliters. For example, if 10 spleens weighing a total of 4 gm. were used, the amount of water added would be 36 ml. Suspensions were homogenized for three to six minutes; cooling was necessary after three minutes of homogenization.

Suspensions made from tissues showing few or a moderate number of leprous lesions and homogenized for three minutes showed well-dispersed bacilli in the smears. Suspensions made from tissues showing very numerous lesions, i.e., at the end of three months, revealed occasional unbroken lepra cells; these specimens were homogenized for six minutes. Longer blending did not materially improve the condition.

Using a 3-mm. platinum loop, duplicate smears of 13 mm. in diameter were made from the original or diluted suspensions. Bacillary counts were made by the use of a 5-mm. square grill in the ocular. One hundred square fields (oil immersion) were examined, covering approximately the whole area of each smear. The bacillary count of an organ was taken as the average number of stainable acid-fast bacilli per square field.

The total number of bacilli in the organ will be the number of bacilli per square field multiplied by the factor of

$$\frac{\text{Area of smear in mm.}^2}{\text{Area of square grill in oil immersion, in mm.}^2} \times \text{No. of loops per ml. of suspension} \times \text{Volume of suspension, in ml.}$$

$$= \frac{132.7}{0.003} \times 220 \times 4, \text{ or approximately } 3.9 \times 10^7$$

for each spleen, and approximately 6.8×10^7 for each liver.

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TABLE 1

THE CELLULAR AND BACILLARY CHANGES IN THE CELLS OF PERITONEAL EXUDATE OF MICE INOCULATED WITH THE KILLED M. LEPRAE MURIUM

Day After	Per Cent Phagocytosis		Per Cent, Number Bacilli per Macrophage				Number Bacilli per 100 Macrophages*	Free Bacilli	Cellular Picture†		
	Polymorphonuclear	Macrophage	1-2	3-5	6-10	>10			Polymorphonuclear	Macrophage	Lymphocyte
									per cent	*per cent*	*per cent*
1	20.9	70.8	16.1	11.7	10.4	61.8	552.4	++	59.4	29.3	11.3
3	0	89.1	6.1	7.4	11.2	75.3	788.0	++	5.0	68.3	26.7
5	0	75.3	11.8	11.2	10.3	66.7	615.8	+	7.1	47.3	45.6
7	0	94.8	11.9	10.3	14.5	63.3	771.7	+	3.9	18.1	78.0
11	0	91.4	14.2	5.3	10.8	69.7	761.4	0	6.0	13.6	80.4
14	0	68.2	32.4	13.5	15.2	38.9	429.3	0	4.6	12.1	83.3
21	0	60.8	45.0	17.2	13.2	24.6	310.3	0	0.3	17.0	82.7
28	0	38.9	51.0	18.2	7.0	23.8	182.4	0	0	13.4	86.6
35	0	18.6	65.9	22.2	5.6	6.3	61.1	+	0	4.9	95.1
42	0	19.8	62.2	16.2	6.7	14.9	77.6	0	0	7.4	92.6
49	0	23.8	68.9	21.4	6.3	3.4	73.3	0	1.6	6.0	92.4
56	0	13.5	75.6	13.3	8.2	2.9	40.4	0	0	4.8	95.2
63	0	16.4	62.7	11.6	13.1	12.6	66.0	0	1.8	4.4	93.8
70	0	10.4	81.9	8.0	5.8	4.3	29.7	0	0.5	3.5	96.0

* The number of bacilli per 100 macrophages was calculated as follows: $(16.1 \times 2 + 11.7 \times 4 + 10.4 \times 8 + 61.9 \times 10) \times 70.8\% = 552.4$.

† The normal cells contain approximately 5 per cent macrophage and 95 per cent lymphocyte.

The Ziehl-Neelsen staining for acid-fast bacilli was modified slightly as follows: The slide was covered with a piece of filter paper. A sufficient quantity of Ziehl-Neelsen acid-fast stain was added and the slide was heated to *boiling*. The smear was decolorized with acid alcohol two minutes later. The high temperature employed seemed to yield more stained bacilli than the original technique.

The cells of the peritoneal smears and mesenteric spreads were stained with Harris' hematoxylin, thirty seconds for the former and ten minutes for the latter, followed by dilute ammonia water.

Growth curves of the infected cells of mesentery and of the bacilli of spleen and liver were plotted on semi-logarithmic paper. Straight lines showing the frequency of infected cells in the mesentery and the bacillary counts for spleen were fitted by the method of least squares by Mr. Jacob E. Lieberman. The generation time (time for one cell division) for bacilli from the spleen was calculated from the logarithmic growth curve in the following manner:[5]

$$K = \frac{\log a - \log b}{t}$$

$$\text{Generation time} = \frac{\log 2}{K}.$$

where K = growth rate constant
a = highest bacillary count
b = lowest bacillary count
t = time in weeks or days

[5] YOUMANS, G. P., AND YOUMANS A. S.: J. Bact., 1949, *58*, 247.

The cells of the peritoneal exudate showed marked changes following inoculation of *M. leprae murium*. In animals which were inoculated with killed bacilli, the polymorphonuclear leukocytes increased to about 60 per cent the day after inoculation. They diminished markedly on the third day and disappeared almost entirely in three weeks. The lymphocytes were only about 10 per cent of the cell population at the beginning; they increased rapidly to about 80 per cent on the seventh day, approaching the normal value of about 95 per cent at the end of five weeks. Mononuclear phagocytes or macrophages increased from about 30 to 70 per cent during the first five days, then decreased gradually, reaching the normal value of about 5 per cent in five weeks (table 1).

The cellular picture differed when living organisms were used. In the early days, the changes of polymorphonuclears and lymphocytes were similar to those observed with killed bacilli. The macrophages, however, maintained more or less a constant level of 20 to 30 per cent throughout the whole experiment. In the later stages, the lymphocytes reached a lower level and the polymorphonuclears a higher one than with the killed bacilli (table 2).

Acid-fast bacilli, living or killed, were found chiefly in the macrophages. Phagocytosis of polymorphonuclears was observed only in the early days. With the killed bacilli the percentages of phagocytosis were very high at the beginning but decreased steadily afterwards. The percentages of

TABLE 2

THE CELLULAR AND BACILLARY CHANGES IN THE CELLS OF PERITONEAL EXUDATE OF MICE INOCULATED WITH THE LIVING M. LEPRAE MURIUM

Day After	Per Cent Phagocytosis		Per Cent, Number Bacilli per Macrophage				Number Bacilli per 100 Macrophages	Free Bacilli	Cellular Picture		
	Polymorphonuclear	Macrophage	1-2	3-5	6-10	>10			Polymorphonuclear	Macrophage	Lymphocyte
									per cent	*per cent*	*per cent*
1	25.9	95.0	4.8	4.3	3.7	87.2	882.0	++	61.3	30.8	7.9
3	+	97.2	1.2	2.6	9.0	87.2	930.0	++	12.0	34.0	54.0
5	+	88.4	1.4	3.1	7.4	88.1	940.1	+	12.1	21.4	66.5
7	0	72.7	12.6	10.1	9.6	67.7	595.7	+	13.2	22.0	64.8
11	0	52.3	36.4	13.2	10.8	39.6	318.0	+	1.0	16.2	82.8
14	0	44.1	39.9	25.0	19.1	16.0	217.2	0	5.7	20.0	74.3
21	0	25.2	62.1	21.5	7.4	9.0	90.6	0	1.0	24.6	74.4
28	0	36.9	49.2	26.1	10.6	14.1	158.2	0	1.4	23.0	75.6
35	0	26.4	59.4	20.5	8.5	11.6	101.6	0	3.6	20.0	76.4
42	0	40.4	41.3	22.0	20.9	15.8	200.3	0	3.0	27.0	70.0
49	0	31.7	56.4	20.8	9.2	13.6	128.6	0	7.8	23.8	68.4
56	0	54.8	35.8	23.3	16.6	24.3	296.2	0	2.0	25.0	73.0
63	0	42.6	42.7	25.7	13.9	17.7	203.0	++	7.6	27.6	64.8
70	0	60.2	36.6	22.0	17.4	24.0	325.3	+	5.6	37.4	57.0

macrophages containing the smallest number of bacilli increased and those containing the largest number decreased steadily throughout the experiment. The number of bacilli per 100 macrophages reached the minimum in five weeks. Living bacilli showed a more rapid decline and reached their lowest value at the end of three weeks, then increased again and fluctuated along a moderately low level throughout the remainder of the experiment.

Extracellular bacilli, both living and killed, were found in the first few days, but were rarely seen after one week, except at later stages in animals which were inoculated with living bacilli. This reappearance was usually accompanied by heavily infected or broken lepra cells, which had apparently separated from the lepromatous lesions in the peritoneal cavity.

Animals which were inoculated with living bacilli but immediately treated with isoniazid, 0.01 per cent in the diet, revealed results similar to those observed with killed bacilli, except that the rate of disappearance of bacilli in the early stage was much faster in the treated animals. The cause of this difference was not determined.

Mesenteric spreads appeared as thin membranes composed of mesothelium with spotty areas of lymphocytes and macrophages. There were also a few scattered macrophages, many of which remained in wandering forms. Marked cellular infiltration was observed in the first five days following infection. The cells were chiefly polymorphonuclears and macrophages, both heavily populated with phagocytized bacilli. A few lymphocytes were present at this stage. Polymorphonuclears and lymphocytes disappeared rapidly and macrophages were dominant at the end of three weeks.

TABLE 3

THE SCORING SYSTEM OF INFECTED CELLS OF MESENTERY

Score	Number of Infected Cells per Square Field	Score	Number of Infected Cells per Square Field
0.5	10	6	160
1	20	7	240
2	40	8	360
3	60	9	500
4	80	10	700
5	100		

Quantitative evaluation of the leprous lesions of the mesentery was made by grading the leprous lesions of the spreads under the low-power objective. Scores of 1 to 10 were given, equivalent to various numbers of infected cells per square field, as shown in table 3. Only cells which were infected with numerous bacilli were taken into account.

There were two main types of lepra cells in the mesentery. Elongated bacilli were present in one, usually the wandering forms, often observed as early as the sixth day of the infection, but more marked on the eighth. The proportion of cells with long bacilli increased steadily to a maximum at the fifth to seventh week, when it decreased abruptly. Mesenteries containing these cells usually showed more leprous lesions than those without them.

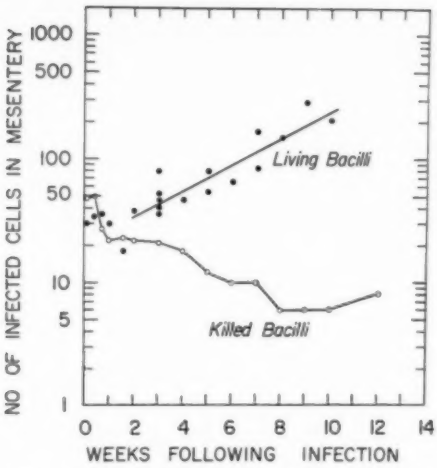

FIG. 1. The growth curves of infected cells of mesentery. The living bacilli curve was constructed from the composite data of five experimental observations at the end of three weeks, two each at the end of five and seven weeks, and one each at various intervals.

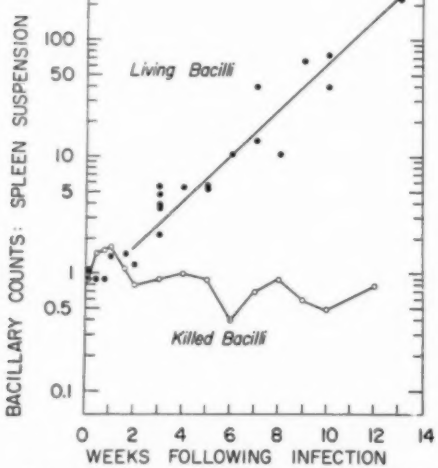

FIG. 2. The growth curves of *M. leprae murium* in the spleen. The living bacilli curves of figures 2 and 3 were constructed from the composite data of five experimental observations at the end of three weeks, two each at the end of five and seven weeks, three at the end of three months, and one each at various intervals.

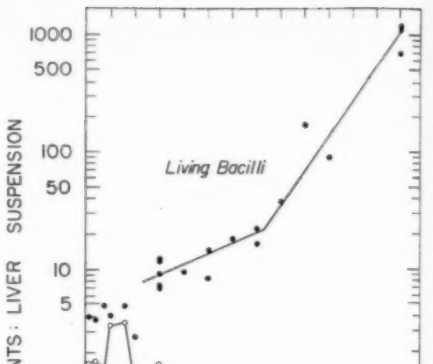

FIG. 3. The growth curves of *M. leprae murium* in the liver. For explanation *see* figure 2.

Apparently, these long bacilli are in active growth. The other type of cell was studded with numerous short bacilli. These were present as isolated cells or in masses of various sizes, with occasional bundle formation. This was the predominant type in the latter stages of the infection.

The curves representing the number of infected cells in the mesentery are shown in figure 1. In animals inoculated with killed bacilli, the number decreased steadily. With the living bacilli, there was an initial decline, followed by a linear logarithmic increase up to the tenth week.

Bacilli were found in the spleen on the day after inoculation of either killed or living organisms. The number of killed bacilli decreased slightly throughout the experiment. The number of living bacilli increased steadily. On semi-logarithmic paper, a linear growth curve was obtained (figure 2). The generation time, as calculated from this curve, was 10.7 days.

The growth pattern of bacilli in the liver was different from that in the spleen. The number of bacilli in the liver increased more slowly than in the spleen in the first seven weeks, but more rapidly afterwards. On semi-logarithmic paper, two linear growth curves were obtained, i.e., a flatter curve followed by a steeper one (figure 3).

Elongation of bacilli was observed in the macrophages of peritoneal smear as early as the fourth

day of the infection. The number of long forms increased rapidly to its maximum in two weeks, and began to decrease after the sixth week of the infection. Elongation of bacilli was also observed in the smears of spleen and liver suspensions. The number of long forms increased steadily, reaching its maximum from five to seven weeks, then decreased rapidly. At the end of three months, short bacilli became dominant, although long forms were still present.

The linear logarithmic growth of bacilli in the spleen of leprous mice is different from that observed in mouse tuberculosis. McCune and Tomsett[9] reported that, in mouse tuberculosis, the splenic census rose rapidly to a maximum, then maintained a slightly lower level for four months. Their animals were infected intravenously.

In the early stage, the growth of *M. leprae murium* is manifested by increase in number and in length of the bacilli; while in the later stages, growth is evidenced chiefly by increase in number. Lengthening, but slower growth, has been reported in rats by Elek and Hilson.[5]

The growth of bacilli in the liver is restricted to some degree in the first seven weeks of the infection. Fite[2] observed that *M. leprae murium* in many instances underwent dissolution in the liver of mice. Disappearance was restricted to the liver and was not uniformly observed. This led him to the conclusion that there is some unknown factor of resistance which is effective for a variable length of time. This would account for the slower growth of bacilli in the early stage of the infection.

Hobby and associates[7] reported a serial study of the bacillary population of spleens of leprous mice. When their data were plotted on semi-logarithmic paper, it was found that the growth was linear from four to six weeks following the infection. From this graph the generation time of bacilli was about 14.8 days, in comparison with 10.7 days in the present study.

Hilson and Elek[10] have reported a logarithmic rate of growth of bacilli in rats and mice which were inoculated intratesticularly with rat leproma. Multiplication was much faster in rats infected with a 7-month-old inoculum than in mice with 18-month-old material. The generation time of bacilli was seven to eight days in rats, and ten days in mice. Similar values of the generation time *in vivo* have been reported recently by other investigators.[11, 12]

In summary: The evolution of murine leprosy was studied in mice inoculated intraperitoneally with the Hawaiian strain of *M. leprae murium*. Animals were sacrificed at one, three, five, seven, eleven, and fourteen days, and thereafter at weekly intervals for ten to thirteen weeks. Four measurements were used: (*1*) number of acid-fast bacilli in the macrophages of the peritoneal exudate; (*2*) number of infected cells in the mesentery; (*3*) estimated bacillary population of the spleen; and (*4*) estimated bacillary population of the liver. The bacilli were taken up rapidly by the cells of the peritoneal exudate and by the cells of the mesentery, spleen, and liver. In the peritoneal exudate, the number of bacilli fell to a minimum in about three weeks, then increased slowly and fluctuated at a moderately low level throughout the experiment. After an initial period of about two weeks following inoculation, the infected cells of the mesentery and the organisms in the spleen followed a more or less constant rate of growth. The bacilli in the liver, however, after the same initial lag period, increased rather slowly to about the seventh week and much more rapidly thereafter. Elongation of bacilli was observed as early as the fourth to sixth day following infection in the macrophages of the peritoneal exudate, and the cells of the mesentery, spleen, and liver. The number of elongated bacilli increased steadily to a maximum at the third to seventh week, and then decreased. Short bacilli became dominant thereafter. The average generation time (time for one cell division) of the bacilli in the spleen was estimated to be 10.7 days.

Y. T. Chang
Leonard Wood Memorial
National Institutes of Health
Bethesda, Maryland

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[9] McCune, R. M., and Tomsett, R.: J. Exper. Med., 1956, *104*, 737.

[10] Hilson, G. R. F., and Elek, S. D.: Internat. J. Leprosy, 1957, *25*, 381.

[11] Rees, R. J. W., and Wong, P. C.: Nature, 1958, *181*, 359.

[12] Hawking, F.: Leprosy Rev., 1958, *29*, 71.

DIFFERENTIATION OF HUMAN TUBERCLE BACILLI FROM ATYPICAL ACID-FAST BACILLI

III. Modification of the Niacin Test, Using Tween-Albumin Liquid Medium

It has previously been shown[1-5] that both virulent and avirulent strains of human tubercle bacilli contain more niacin than bovine or avian strains or other species of mycobacteria, including the so-called "atypical acid-fast bacilli." Tubercle bacilli of human origin can therefore be differentiated from other mycobacteria by measuring niacin content. These results were confirmed by others.[6-9]

However, most clinical specimens grown on routine diagnostic media do not yield enough growth for a satisfactory interpretation of the niacin test.[3] In these cases a subculture is necessary, the subculture period usually being one to two months; and even after subculture enough growth may not be obtained.

In this paper it will be shown that, by growing mycobacteria for about ten days in Dubos Tween®-albumin liquid medium,[10] a positive test for niacin can be obtained. Thus, a subculture period of ten days is sufficient for a satisfactory niacin test. An additional finding revealed that niacin content increases with older cultures.

Niacin was measured by the aniline cyanogen bromide method[11] which is based on the reaction of niacin and cyanogen bromide to give pyridinium compound. This compound undergoes a rearrangement yielding amines to produce yellow-colored compounds.

The organisms tested are shown in table 1. Ten strains of tubercle bacilli of human origin and 10 strains of atypical acid-fast bacilli were used. These strains had been cultured repeatedly in Dubos liquid Tween-albumin medium. One-tenth milliliter of a ten-day culture was inoculated into 3 ml. of Dubos Tween-albumin liquid medium in 20 by 125 mm. screw-capped tubes. Growth was roughly estimated by comparison with McFarland nephelometer tubes. Niacin content was measured by adding 1 ml. of 4 per cent aniline in 95 per cent ethyl alcohol and 1 ml. of a 10 per cent solution of cyanogen bromide in water (saturated solution) directly into the culture tube; the development of a distinct yellow color indicated the presence of niacin.

For quantitative measurements, 1 ml. instead of 0.1 ml. of culture served as an inoculum, and 100 ml. of medium in a 250-ml. Erlenmeyer flask was used. The culture flasks were shaken daily. At ten-day intervals for thirty days, the cultures were centrifuged and niacin determinations were performed in the supernatant, using ammonia buffer and cyanogen bromide solution.[2] The color intensity was measured in a Bausch & Lomb monochromatic colorimeter at 430 mμ. Three strains of human tubercle bacilli, one strain of bovine tubercle bacilli, one strain of avian tubercle bacilli, 3 strains of atypical mycobacteria, and 2 strains of nonpathogenic mycobacteria were used.

When dealing with the scotochromogenic atypical acid-fast bacilli,[12] the yellow or orange pigments of the bacilli dissolve in the medium, making the test more difficult to read. In this case a portion of the medium was divided equally into two tubes and both reagents were added to one of the tubes. One milliliter of the aniline solution and an equal amount of water were added to the other tube. This tube served as a control.

The results presented in table 1 show that, of the strains examined, only the tubercle bacilli of human origin gave a positive test for niacin. The test was definitely positive after ten days of growth. At this time growth was roughly comparable to a McFarland No. 2 tube (about 0.85 mg. wet weight of H37Rv). All strains of atypical acid-fast bacilli were negative for niacin in spite of the fact that growth of some strains was more abundant.

The quantitative differences in niacin production of various strains of mycobacteria are shown in table 2. It can clearly be seen that the tubercle bacilli of human origin produce more than fifteen times as much niacin as the other mycobacteria except for the Ravenel strain of bovine tubercle bacilli, which produces a small amount of niacin. It appears that with the human mycobacteria

[1] Konno, K.: Proc. Japan Acad., 1953, *29*, 289.
[2] Konno, K., *et al.*: Am. Rev. Tuberc., 1957, *75*, 529.
[3] Konno, K., *et al.*: Am. Rev. Tuberc., 1958, *77*, 669 and 675.
[4] Konno, K.: Science, 1956, *124*, 985.
[5] Konno, K.: Beitr. Klin. Tuberk., 1958, *119*, 1.
[6] Marek, A.: Beitr. Klin. Tuberk., 1957, *117*, 529.
[7] Boenicke, R.: Jber. Tuberk. Forsch., 1956/1957, p. 137.
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[9] Jensen, K. A.: Bull. Union internat. contr. tuberc. Paris, 1957, *27*, 147.
[10] Dubos, R. J., and Middlebrook, G.: Am. Rev. Tuberc., 1947, *56*, 334.
[11] Feinstein, L.: Science, 1945, *101*, 675.
[12] Runyon, E. H.: Am. Rev. Tuberc., 1955, *72*, 866.

TABLE 1

NIACIN TEST IN DUBOS LIQUID MEDIUM (3 ML.)

	Culture Period	0 day	2 days	4 days	6 days	8 days	10 days	Positive Total Strains
10 strains of tubercle bacilli of human origin	Niacin test, positive	0	0	0	2	3	5	10
	Growth in McFarland tube No. 2	0	0	0–1	1–2	2–3	2–3	
10 strains of atypical acid-fast bacilli	Niacin test, positive	0	0	0	0	0	0	0
	Growth in McFarland tube No. 2	0	0	0–1	1–2	1–3	2–4	

TABLE 2

NIACIN PRODUCTION OF MYCOBACTERIA IN DUBOS LIQUID MEDIUM (100 ML.)

Types	Strain	10 days			20 days			30 days		
		Bacilli Dry Weight	Niacin Filtrate	Niacin Bacilli	Bacilli Dry Weight	Niacin Filtrate	Niacin Bacilli	Bacilli Dry Weight	Niacin Filtrate	Niacin Bacilli
		mg.	*γ/ml.*	*γ/mg.*	*mg.*	*γ/ml.*	*γ/mg.*	*mg.*	*γ/ml.*	*γ/mg.*
Human tubercle bacilli	H37Rv	36	3.2	8.9	105	12.5	11.9	122	18.0	14.8
	H37Ra	28	2.6	9.3	78	8.0	10.2	103	11.2	10.9
	RIRv	30	2.3	7.7	90	9.8	10.9	110	14.5	13.2
Bovine tubercle bacilli	Ravenel	30	0.5	1.7	88	1.8	2.0	111	2.7	2.4
Avian tubercle bacilli	Kirchberg	47	0.2	0.4	112	0.3	0.3	132	0.6	0.5
Atypical acid-fast bacilli	M.B.	33	0.2	0.6	82	0.3	0.4	116	0.6	0.5
	T.N.	37	0.2	0.5	97	0.3	0.3	123	0.6	0.5
	M. Kansasii	36	0.2	0.5	89	0.3	0.3	116	0.6	0.5
Nonpathogenic acid-fast bacilli*	*M.* 607	61	0.5	0.8	120	0.6	0.5	144	0.8	0.5
	M. phlei	66	0.5	0.8	124	0.8	0.6	145	1.2	0.8

* Culture periods for nonpathogenic acid-fast bacilli were five, ten, and fifteen days.

niacin production increases with the age of the culture.

* * *

Tubercle bacilli of human origin will give a positive test for niacin after about ten days of growth in Dubos Tween-albumin liquid medium. Konno and associates[2] have shown that about half of the routine cultures brought to a clinical laboratory for identification needed a subculture period of at least one month before sufficient growth was obtained for a satisfactory niacin test. By using Dubos Tween-albumin liquid medium as the subculture medium, it was apparent that the subculture period was shortened to about ten days.

The quantitative data[3] show that about 10 mg. of semi-dry bacilli are necessary before a positive test for niacin is obtained. Marek[6] from his results believes that it is safer to raise this figure to 20 mg. With increasing bacillary material, an increased niacin content is noted. This finding may have at least two different explanations: first, the organisms may be producing more niacin with increasing age; or second, the organisms with increasing age are breaking down diphosphopyridine nucleotide (and/or triphosphopyridine nucleotide) to a greater extent, due to the presence of a more active DPN-ase and/or TPN-ase. More work directed toward the elucidation of this phenomenon is in progress. Also, it was noted that niacin production in Dubos liquid medium is much greater than on solid medium. The amount of niacin produced in Dubos liquid medium is about 10γ per mg. of dry bacilli compared with 0.15γ per mg. of semidry bacilli on solid medium.[3] It is more advantageous, therefore, to use Dubos medium for the niacin test than to subculture on solid medium.

In contrast, no significant increase in niacin

content of tubercle bacilli of bovine or avian origin was noted. The bovine strain, when it showed eugonic growth, produced slightly more niacin than other non-human mycobacteria and occasionally gave a doubtful positive test for niacin in Dubos medium. In this case, Boenicke and Lisboa[8] recommended use of the Peknice reaction[13] or the Asmus-Garschagen reaction[14] which are less sensitive than the aniline-brom-cyanid method for the estimation of niacin in liquid medium. But the aniline-brom-cyanid method should be used for a niacin test on the solid medium, because niacin production of the bacilli on the solid medium is much less than in liquid medium[3] and, therefore, the more sensitive method is reliable.

[13] Pecknice, J. R.: Čsl. Farmac., 1953, *2*, 354.

[14] Asmus, E., and Garschagen, H.: Ztschr. Anal. Chem., 1953, *139*, 81.

[15] The writers wish to express their appreciation to the Cambridge Sanatorium, Cambridge, Massachusetts, for aid and support of this study.

In summary: It has been shown that, among 20 strains of mycobacteria grown in Dubos Tween-albumin liquid medium, only tubercle bacilli of human origin gave a positive test for niacin. The aniline-cyanogen bromide test for niacin was definitely positive after ten days of growth in Dubos Tween-albumin liquid medium.

The niacin production in Dubos liquid medium was much greater than on solid medium, and it is thus more advantageous to use Dubos liquid medium for the test.

Kiyoshi Konno[15]
Anthony J. Sbarra
Research Institute for Tuberculosis and Leprosy
Tohoku University
Kitayobancho, Sendai, Japan; and
Department of Bacteriology and Immunology
Harvard University Medical School
Boston, Massachusetts

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THE EFFECTS OF MECHANICAL AGITATION ON THE GROWTH OF THE H37Ra STRAIN OF M. TUBERCULOSIS

Lenert, Stasko, and Hobby[1] recently described in this journal a method of obtaining large yields of the BCG strain of *M. tuberculosis* by cultivation under continuous agitation on a rotary shaker. The medium employed by Lenert and her coworkers was a synthetic one, consisting of sodium glutamate, citric acid, glycerol, dibasic potassium phosphate, ferric ammonium citrate, and magnesium sulfate. The organisms were grown in 1-liter quantities of medium in Fernbach flasks; each flask was inoculated with 100-ml. portions of a young, stationary Tween®-albumin medium seed culture, and placed on the shaker, operating at 200 r.p.m., for various periods of time. Under these conditions, yields of a dried bacillary mass averaging 19.45 gm. per liter were obtained after an incubation period of fourteen days on the shaker, or twelve days' stationary growth, with manual shaking once daily, followed by eleven days on the shaker.

Working in the laboratories of the Rockefeller Institute for Medical Research, the writer in 1953 observed a similar enhancement of growth by incubation on a rotary shaker of another strain of tubercle bacilli, H37Ra, employing somewhat different conditions of cultivation. These experiments have not been reported previously, and a brief report is made here to indicate the applicability of shaker-cultivation for the production of large yields to another strain of tubercle bacilli, under different conditions than those reported by Lenert and associates.

The H37Ra strain employed in these experiments has been described previously by Pierce and associates[2]; its growth in the conventional Tween-albumin and Sauton liquid media is sparse when cultivation is stationary, with only occasional manual agitation.

The medium was the standard basal mineral solution described by Dubos and Middlebrook,[3] containing, in addition to the inorganic salts, 0.2 per cent asparagin, 0.005 per cent ferric ammonium citrate, 0.013 per cent sodium citrate, 1.5 per cent glycerol, 0.05 per cent N-Z Case (Sheffield), and 0.1 per cent bovine serum albumin fraction V (Armour); Tween-80 (Atlas) was added to some lots of this medium to a final concentration of 0.05 per cent. The pH was adjusted before autoclaving to 6.8 with hydrochloric acid and sodium hydroxide; it was found to vary by not more than 0.1 pH unit after sterilization. The medium was distributed in 50-ml. quantities in 250-ml. Erlenmeyer flasks, and inoculated with 2.0-ml. aliquots of a 7-day-old culture of the organism grown statically on the same Tween-containing medium. The flasks were then incubated at 37° C., either statically with a one-minute period of manual shaking daily, or on a New Brunswick gyrotory shaker at 80 r.p.m. (A fan was attached to the flywheel of the shaker to dissipate the heat generated by the motor; when this was done, the temperature of the medium did not vary more than ±0.5° C. from that of the statically incubated cultures.) At intervals after inoculation, the cultures were killed by the addition of liquid phenol (88 per cent) to a final concentration of 2 per cent. After standing in phenol six to eight hours with frequent manual shaking, the cultures were pooled and the bacillary mass was separated from the medium by centrifugation and washed five times with large quantities of cold acetone and distilled water by resuspension and centrifugation. The mass was then dried to constant weight over anhydrous calcium chloride and weighed. The results of a representative experiment are shown in table 1 in which 10 flasks containing 50 ml. each of medium, with and without 0.05 per cent Tween, were inoculated with H37Ra and incubated for fifteen days, statically or on the shaker.

It may be seen from table 1 that there was an approximately ninefold increase in the yield of phenolized, washed, and dried tubercle bacilli grown on the shaker, vis-a-vis statically incubated cultures; the increase in yield was proportionately the same whether Tween was present or absent in the medium, but the total yield was greater with Tween. In an experiment in which the same organism was grown for twenty-five days in a similar but less rich medium, containing only 1 per cent glycerol, but also 0.3 per cent ammonium sulfate and 0.005 per cent sodium oleate, the yields per liter of statically and shaker-incubated cultures were, respectively, 0.84 and 2.76 gm.

The growth-enhancing effects of continuous shaking were most marked when a period of seven to fifteen days' static incubation preceded cultivation on the shaker. Alternately, almost equally large yields were obtained by seeding cultures very heavily—the usual procedure here was to inoculate media with one-tenth their volume of a 7-day-old Tween-albumin culture—and placing them on the shaker from the start. With small inocula, immediate cultivation on the shaker

[1] Lenert, T. F., *et al.*: Am. Rev. Tubere., 1958, *78*, 934.

[2] Pierce, C. H., *et al.*: J. Exper. Med., 1953, *97*, 189.

[3] Dubos, R. J., and Middlebrook, G.: Am. Rev. Tubere., 1947, *56*, 334.

TABLE 1

YIELDS* OF PHENOL-KILLED, WASHED, AND DRIED TUBERCLE BACILLI (H37Ra) GROWN FOR 15 DAYS IN STATIONARY AND SHAKEN CULTURES

Medium	Cultivation of the Bacilli	
	Stationary	Shaken
	gm.	*gm.*
Dubos medium, no Tween	0.275	2.250
Dubos medium, +0.05 per cent Tween	0.330	3.020

* Pooled yields from a total of 500 ml. of medium, distributed in 50-ml. quantities in 250-ml. Erlenmeyer flasks.

actually retarded growth for several days, and the final yield was never as great. It thus appeared that a considerable bacillary mass must be present in cultures at the moment at which continuous agitation is begun in order to obtain a large yield of bacilli rapidly. However, even when seeding was done with relatively small inocula, of the order of one-fiftieth the volume of the production media, the yields were still consistently and considerably greater after two weeks or longer incubation than those obtained from stationary growths.

When the bacilli were cultivated on the shaker immediately after seeding with small inocula, in media containing no Tween, they usually grew in the form of discrete pellets similar to those seen in shaken cultures of many species of streptomyces. The bacilli appeared to be morphologically normal, but a much greater number were found to be nonacid-fast, or only weakly acid-fast, than bacilli grown statically.

It also proved possible to obtain good yields of H37Ra bacilli by growing them in "deep" culture under continuous or intermittent agitation and aeration:

The Dubos medium described above, containing 0.5 per cent glycerol and 0.1 per cent bovine serum albumin fraction V, but no Tween, was distributed in 1- to 2-liter quantities in 3-liter side-arm flasks, and inoculated with 20 ml. of a 7-day-old Tween-albumin seed culture. The flasks were incubated at 37° C., over a magnetic stirrer which agitated a Teflon-coated magnetic bar in the medium, either continuously or for three to four periods of ten to thirty minutes each, daily. Air sterilized by passage through concentrated sulfuric acid and cotton filters was drawn through a capillary tube in a fine stream over the surface of the medium by means of a vacuum arrangement; aeration was maintained for eight continuous hours each day.

The yields obtained by this method, which has the advantage of providing a homogeneously dispersed growth of tubercle bacilli in large volumes in a single vessel and without the addition of complex organic wetting agents, were not as great as those resulting from incubation on the shaker, but were still several times greater after two to three weeks' incubation than those given by static cultures.

Similar findings were made when the BCG-Tice strain, which also grows sparsely in most media when cultivated statically, was grown in "deep" culture with frequent agitation and aeration. (It should be noted that no attempt was made in this, or in the shaker-cultivation, experiment to determine the *best* medium. The media employed were rather those used routinely for different purposes in the laboratory, and undoubtedly still better yields would have been obtained from agitated cultures if the optimal concentrations of medium constituents had been empirically ascertained.)

TABLE 2

EFFECT OF TWEEN, ACETATE, LACTATE, AND GLYCEROL ON THE GROWTH OF H37Ra IN STATIONARY AND SHAKEN CULTURES

Addition to Basal Dubos Medium, Containing 0.05 Per Cent Tween	Growth* of H37Ra After 13 Days' Incubation in	
	Stationary Cultures	Shaken Cultures
per cent		
Additional Tween to 0.1	++	++
Additional Tween to 1.0	++	+
Sodium acetate to 0.1	++	++
Sodium acetate to 0.3	++	0
Sodium acetate to 0.5	0	0
Sodium acetate to 1.0	0	0
Sodium lactate to 0.1	++	++
Sodium lactate to 0.3	++	+
Sodium lactate to 0.5	++	+
Sodium lactate to 1.0	+	0
Glycerol to 0.2	++	+++
Glycerol to 0.5	++	+++
Glycerol to 1.0	++	++++
Glycerol to 3.0	+	++++
Glycerol to 6.0	+	++++

* Growths estimated on pooled flasks (2) of each medium by visual grading:

0 = no growth
+ = slight growth
++ = considerable growth
+++ = heavy growth
++++ = luxuriant growth

It appeared early in the course of these experiments that, whereas shaken cultures produced much larger yields of H37Ra in the usual laboratory media, the opposite was true of certain other media, especially those containing larger quantities of Tween or of certain organic acids. An experiment was therefore initiated to determine the effects of the addition of Tween, acetate, lactate, and glycerol to the standard Dubos medium on the growth of H37Ra in stationary and shaken cultures.

Dubos medium, containing 0.2 per cent bovine serum albumin fraction V and 0.05 per cent Tween-80, was distributed in 50-ml. quantities in 250-ml. Erlenmeyer flasks. To 4 flasks each of this medium, one of the following substances was added: additional Tween, to a final concentration of 0.1 or 1.0 per cent; sodium acetate, to concentrations of 0.1, 0.3, 0.5, and 1.0 per cent; sodium lactate, to similar concentrations; and glycerol, to concentrations of 0.2, 0.5, 1.0, 3.0, and 6.0 per cent. The pH of all media was adjusted to 7.0. After sterilization by autoclaving, the media were inoculated with 1-ml. aliquots of a stationary, 7-day-old Tween-albumin seed culture of H37Ra. Two flasks of each medium were incubated statically at 37° C., with one minute of manual agitation daily, and two on the gyrotory shaker at 80 r.p.m. at the same temperature. Thirteen days later, the amount of growth in the different flasks was estimated visually. The results are shown in table 2.

It may be seen from table 2 that the growth of the organism on media containing 0.05 per cent Tween and glycerol from 0.2 to 6.0 per cent was greater when cultivation was on the shaker. Conversely, larger amounts of Tween, and acetate and lactate proved inhibitory to the organism in the agitated cultures at concentrations which still permitted static growth. Similar observations were made employing the BCG-Phipps and R1Rv strains, Tween, acetate, and lactate proving inhibitory at lower concentrations when the organisms were cultivated on the shaker at 80 to 100 r.p.m., or on a tissue-culture test-tube roller at 2 r.p.m., than when they were grown statically. These findings suggest that the effects on growth of tubercle bacilli of certain medium constituents and common metabolites may vary widely with the degree of agitation (and aeration?) of the cultures.

In summary: It appears possible to obtain large quantities of the H37Ra strain of *M. tuberculosis* rapidly, and probably also of other strains, by cultivation under frequent or continuous mechanical agitation. This can be accomplished by growing the organisms on a shaker, or in "deep," aerated cultures stirred magnetically. Large yields of well-dispersed growth can be obtained in the standard Dubos albumin medium, with or without the addition of Tween. The only other method of obtaining large yields of tubercle bacilli by surface-pellicular growth requires considerably more time and produces a nonhomogeneous bacillary mass, much of which is nonviable at the time of harvesting. In the production of nonliving vaccines from tubercle bacilli, the agitation methods described above and by Lenert and associates[1] are not only desirable for their economy and convenience, but also because homogeneity of the starting material is a primary requisite for the obtaining of reproducible results in immunization experiments.

DAVID W. WEISS
Department of Bacteriology
University of California
Berkeley 4, California

January 19, 1959

A NEW METHOD OF COUNTING THE VIABLE ORGANISMS IN BCG VACCINE

Considerable differences in the count of viable organisms in a BCG vaccine can be obtained between and within laboratories even though the medium and the procedures used have been standardized. The most significant reason for the discrepancies is probably the medium employed. Most tuberculosis media are complex preparations, and even slight variation in their composition may influence the results. To overcome this, attempts were made to produce a simple reproducible method which would yield consistent results. A number of media were compared. The most successful proved to be solid medium overlaid with fluid using Tarshis blood medium without penicillin[1] as the solid base and Sauton medium with buffer and human serum[2] as the fluid. The results obtained with this medium were so encouraging that the writers have been prompted to report the procedure.

Media and procedure:

(a) Solid base (Tarshis medium):

Agar	15 gm.
Glycerol (analytic grade)	10 ml.
Human bank blood	250 ml.
Distilled water	740 ml.

The agar is dissolved in the glycerol water. The mixture is autoclaved at 15 pounds' pressure for fifteen minutes and cooled to 45° C. The blood is added aseptically and the medium is dispensed in 25 to 30 ml. quantities in 250-ml. Erlenmeyer flasks. The flasks are plugged with cotton and covered with paper, secured by a rubber band, to control the rate of evaporation.

(b) Sauton medium with buffer and serum:

(*1*) Sauton medium

Asparagin	4 gm.
Magnesium sulfate	0.5 gm.
Citric acid	2.0 gm.
Glycerin (twice-distilled)	60.0 gm.
Bipotassium phosphate	0.5 gm.
Ferric ammonium citrate	0.05 gm.
Distilled water	940 ml.

The medium is adjusted to pH 7.2 with ammonium hydroxide and autoclaved at 15 pounds' pressure for twenty minutes.

(*2*) Buffer (pH 7.15)

$Na_2HPO_4 \cdot 12H_2O$ (179 gm. in 1,000 ml.)	670 ml.
KH_2PO_4 (68 gm. in 1,000 ml.)	330 ml.

The final mixture is prepared by adding 800 ml. of distilled water and 200 ml. of the buffer (solution 2) to 1 liter of Sauton medium (solution 1). This is autoclaved at 15 pounds' pressure for twenty minutes. After cooling, sterile human serum is added aseptically to a concentration of 5.0 per cent and the medium is dispensed in tubes in 9-ml. quantities.

The counting is carried out as follows: The vaccine is shaken thoroughly on a wrist-action shaker, set at 250 to 300 oscillations per minute, for thirty minutes, and serial dilutions are made to 10^{-7} using Sauton diluent (1 part Sauton medium and 3 parts distilled water). One milliliter of the 10^{-7} dilution is then added to the tubes containing 9 ml. of the buffer Sauton complex (solution (b) above). This is then shaken on a mechanical shaker (either a wrist-action shaker set at 250 to 300 oscillations per minute or a reciprocating type of shaking apparatus set at 200 to 225 oscillations per minute) for two and one-half hours, being protected from light by aluminum foil wrappings. Using aseptic technique, it is then poured over the solid blood medium in the Erlenmeyer flasks. These are then incubated at 37° C. for three weeks. Movement of the flasks must be strictly avoided for the first two weeks to prevent the break-up of growing colonies and clumps.

The liquid evaporates or is absorbed into the medium within ten days. The colonies make their appearance about the twelfth day, and in twenty-one days are well separated, distinct, circular, raised, light brown in color, and the majority are rough.

Results: This method was compared with six others, which included four combinations of solid medium overlaid with fluid (Tarshis medium overlaid with either (*a*) Sauton medium, (*b*) Sauton medium with human serum, (*c*) Sauton medium plus 1 per cent charcoal, or (*d*) Dubos liquid medium) and two solid media (Dubos oleic-acid-agar and Tarshis blood-agar). The medium described here, Tarshis medium overlayed with Sauton medium with buffer and human serum, in general yielded the highest count and gave the most consistent counts.

An example of the reproducibility of this method and a comparison with the previous standard method employed in this laboratory (Dubos oleic-acid–agar) is shown in table 1, in which the results of counts of 5 fluid and 5 dried vaccines, selected at random, are shown. The individual counts of each flask and plate are shown in the table as well as the average number of viable organisms in each vaccine. As mentioned above, the spread of results within a test is generally less than that obtained with the other

[1] Tarshis, M. S., and Elberg, S. S.: Am. Rev. Tubere., 1956, *74*, 84.

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TABLE 1

COMPARISON OF COUNTS OF BCG VACCINE WITH A SOLID MEDIUM OVERLAID WITH FLUID MEDIUM AND DUBOS OLEIC-ACID-AGAR MEDIUM

Vaccine Number	Colony Count × 10^6			
	Solid Medium Overlaid with Fluid Medium—5 Flasks	Average	Dubos Oleic-Acid-Agar Medium—5 Plates	Average
Fluid				
1	390, 400, 410, 490, 540	446	230, 300, 300, 320, 460	322
2	440, 480, 520, 540, 550	506	330, 370, 420, 430, 580	426
3	500, 520, 540, 560, 670	558	370, 380, 400, 400, 410	392
4	420, 570, 570, 620, 670	570	370, 420, 430, 440, 480	428
5	190, 290, 290, 290, 310	274	250, 260, 310, 340, 350	302
Dried				
1	1.6, 1.6, 1.7, 1.8, 2.1	1.7	1.4, 1.6, 1.7, 1.9, 2.0	1.7
2	3.1, 3.3, 3.7, 3.9, 4.0	3.6	0.9, 1.0, 1.0, 1.1, 1.2	1.0
3	500, 560, 600, 620, 640	584	400, 400, 500, 560, 560	484
4	120, 120, 180, 180, 200	160	80, 100, 100, 160, 180	124
5	360, 480, 480, 500, 560	476	400, 400, 500, 520, 580	480

media used, and the average count is usually higher.

* * *

The advantages of solid medium overlaid with fluid may be listed as follows: The blood agar surface is never completely dry, resulting in better colony appearance. The results are more uniform and the counts are generally available sooner than with other media. The colonies are distinct, well separated, and easy to count. The buffered Sauton medium reduces or eliminates bacterial contaminants.

The Tarshis blood-agar medium would appear to be the medium of choice as the solid base of any combination of media since, in addition to its being excellent for the growth of mycobacteria, its color allows for more distinctive colony formation than other media. Furthermore, the presence of hemin might be a help in the isolation of organisms weakened by exposure to isoniazid.

The procedure reported here has interesting potentialities in diagnostic work and in identification of mycobacteria.

B. B. DIENA
L. EIDUS
L. GREENBERG
Biologics Control Laboratories,
Laboratory of Hygiene
Department of National Health and Welfare
Ottawa, Canada

February 3, 1959

Correspondence

CHEMOTHERAPY IN PNEUMOCONIOSIS COMPLICATED BY TUBERCULOSIS

To the Editor of the American Review of Tuberculosis and Pulmonary Diseases:

We are perturbed by the poor results which have been reported with chemotherapy in pneumoconiosis complicated by open tuberculosis. The article by Dr. Morrow and Dr. Kantor in your issue of October, 1958[1] stimulated us to a review of the literature and to an analysis of our own results. All detailed reports, including the most recent ones,[1-5] stress the infrequency of sputum conversion to negative in these cases. Resistance often emerges, and the prognosis is regarded as inevitably gloomy.

Our results are much more encouraging. We investigated all patients with pneumoconiosis and with sputum positive on culture for tubercle bacilli who had been admitted to three Scottish hospitals from 1953 to 1958. There were 25 such patients, mostly coal miners. In 18 the presence of progressive massive fibrosis indicated severe pneumoconiosis. In 12, streptomycin (1 gm.), PAS (10 to 20 gm.), and isoniazid (200 mg.) were given daily. In 8, the chemotherapy was similar except that the streptomycin was given three times weekly. Two were given streptomycin, 1 gm., and isoniazid, 200 mg., both daily: one, streptomycin, 1 gm., three times weekly with PAS, 15 gm. daily; and one, PAS, 10 gm. daily, with isoniazid, 200 mg. daily. In every patient there was sputum conversion to negative culture within the first three months. One patient relapsed after five months because he refused further treatment. In another patient, in whom streptomycin had to be withdrawn because of toxicity, a positive culture was obtained in the eighth month of treatment though PAS and isoniazid were being given; viomycin was given in addition, and all subsequent cultures have remained negative. In the remaining 23 patients the sputum has been consistently negative on culture. After discharge, the patients have continued with combined cachets containing a daily dose of 10 gm. of PAS and 200 mg. of isoniazid. The average duration of chemotherapy was two years. So far none has relapsed, although in half of the patients chemotherapy has been stopped for a mean period of nine months.

Pretreatment sensitivity tests showed full sensitivity to all three drugs, except for streptomycin in one instance. No resistance emerged during treatment. The roentgenographic lesions usually regressed only slightly, and cavity closure was uncommon.

Fuller details will be given in a paper which we hope to publish shortly. In the same report, we have also tried to account for the failure encountered by so many others. Our results do show that, by using principles developed in uncomplicated pulmonary tuberculosis,[6, 7] sputum conversion to negative should be achieved in almost all patients who suffer from advanced pneumoconiosis complicated by open tuberculosis.

A. Pines[8]
J. H. Rolland Ramsay
Department of Tuberculosis and Diseases of the Respiratory System
Edinburgh University and
Tuberculosis and Respiratory Diseases Unit
Bangour Hospital
Scotland

February 27, 1959

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[5] Soloveva, V. A.: Probl. Tuberk., 1957, *35*, 62.

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[8] We are indebted to Drs. G. J. Summers, C. Kelman Robertson, N. W. Horne, I. W. B. Grant, and Professor J. W. Crofton for allowing us to investigate their patients and for criticism.

Books

LA SOUCHE DU BCG. By A. Frappier and M. Panisset. Pp. 120, Monograph of the Institute of Microbiology and Hygiene of the University of Montreal, 1957.

This book, written by investigators who have been studying BCG for more than twenty-five years, concerns the work of several international committees: The Committee of Experts on Tuberculosis of the O.M.S., The Commission on BCG of the International Antituberculosis Union, and the International Childhood Center. The authors draw, not only on a very complete historic study of the original strain of BCG, but also on the comprehensive inquiries they have made at forty-eight laboratories producing BCG.

Since BCG was first administered to the infant in 1924, approximately fifty laboratories throughout the world have been preparing vaccinal suspensions of BCG. Some institutions use strains periodically received from the Pasteur Institute or from other central laboratories (Danish, Swedish, Brazilian), and others employ the strain that the Pasteur institute gave them twenty to thirty years ago. In the various laboratories, the stocks are not kept in a rigidly uniform fashion, nor is the vaccine prepared in a standard way. It is therefore not surprising that vaccines supplied by different laboratories sometimes vary in their allergizing properties or in the frequency of the lymphatic tissue reactions they provoke in man.

Are these variations due to the method of preparation or to the way of using the vaccinial suspension, to phenotypical variations, or, on the contrary, to the creation of new substrains differing genotypically from the original stock of Calmette and Guerin? The two mechanisms first mentioned unquestionably have important influences, but are easily rectified. Consequently, it is the latter possibility which the authors examined with the greatest care and present under the title: "Has the strain of BCG varied since 1933?"

Between 1921 and 1923, Calmette and his school described the pathogenic, allergizing, and immunizing properties of BCG. The description, very accurate for that time, is carefully analyzed by Frappier and Panisset, who conclude that, if actual substrains of BCG are studied with the same technique, most of them are found to be strikingly similar to the original one studied by Calmette.

Between 1924 and 1930, the principal concern was to check for the eventual return of virulence. Since 1930, this fear has not materialized but, instead, "over-attenuation" has been a particular concern among clinicians. In fact, if Calmette's testing techniques are applied to the guinea pig, this fear is not justified, even in 1958.

On the other hand, if different substrains of BCG are examined with modern techniques (intradermal inoculation in the guinea pig, intravenous in the mouse), it becomes apparent that the substrains are not rigorously identical and may differ in certain genotypic characteristics. *In vitro*, they may differ in their ability to grow in Besredka's medium, in their reaction to neutral red, in their reaction to sodium salicylate (*see* the work of the Commission on Laboratory Methods of the International Antituberculosis Union), or in giving rise to "spreading" or "nonspreading" colonies (Dubos). In *in vivo* systems, they may differ in their ability to multiply and survive in mouse tissues, an aptitude which generally parallels their immunizing power for this animal (Dubos and his school).

The fact that different substrains of BCG actually exist proves that the original strain has been able to change in various ways in some laboratories. *The possibility of these variations makes it mandatory to have a constant control of BCG*, and the authors devote the second part of their book to such a control. The control consists in determining in the laboratory, before using it in man, whether a substrain is suitable for human vaccination, and eventually to make the choice among the substrains in actual use. Unfortunately, the parallel between the results of laboratory tests and the influence of BCG in man is still far from constant. In some exceptional cases, correlation exists: the Phipps strain corresponds in every respect (high proportion of "spreading" colonies, vaccinating effectiveness in the mouse, aptitude to provoke adenopathies in man) to the strains "Moreau" or "Tyce." In other instances, however, the parallel is far from proved and, according to the authors, the most urgent task for investigators of BCG is to determine the most suitable laboratory tests for the effective control of substrains of BCG as well as for vaccinal suspensions themselves.

This book does not claim to solve the problem of control, but rather to chart a work program for organization of the setup for a control. The authors stress the difficulties of this work, the strict

conditions heretofore uncommonly adhered to, in which comparisons must be made not only of vaccines ready for use, but also of substrains.

Although in the limited space of a 120-page book, which is very well edited and excellently produced, the authors unfortunately cannot make a very critical study of the literature, nor sufficiently review the causes of error in some of the techniques essential to any control, e.g., the enumeration of viable bacilli, this work will be an indispensable guide to all who wish to become familiar with the problem of the control of BCG.

NOEL RIST
Paris, France

REHABILITATION AFTER ILLNESS AND ACCIDENT. Edited by Thomas M. Ling and C. J. S. O'Malley. Pp. 119, The Williams and Wilkins Co., Baltimore, 1958. Price, $3.50.

This short and extremely readable book takes a refreshingly frank look at what rehabilitation is and why some doctors are interested in it and others are not. Written by British authors, it necessarily relates rehabilitation to a more inclusive social coverage than exists in the United States, and clearly shows that there are liabilities connected with this as well as assets.

The introduction of the book is an excellent summary of the general principles of rehabilitation in its broadest sense. It points out that rehabilitation as the doctors' responsibility can only be done with team-work, and is becoming increasingly recognized as an integral part of any hospital service.

The chapter by Dr. Ling on emotional factors in rehabilitation has such simplicity and clarity that it can immediately be used by any doctor or by nursing staffs. This keynote of practicability extends throughout the book and makes it much more usable than philosophic or highly specialized texts.

In Dr. Chiesman's chapter, he takes the provocative problem of increased industrial absenteeism in spite of all our medical advances, and very forthrightly argues the factors involved. Particularly interesting is his belief that definitions of positive health make people apprehensive about their present condition which, of course, is the beginning of ill health.

Dr. Davies, who is the Professor of Social Medicine at the University of Malaya in Singapore, expresses some bitterness in his chapter on rehabilitation after illness. He feels the role of the industrial medical officer is honestly quite limited and that rehabilitation is only important when there is full employment. He does express the importance of doing a full profile of the patient's physical, mental, emotional, social, and vocational factors before making a decision about return to work. He cautions against sympathy which he says is a grossly overworked word and "covers oceans of slush."

Dr. John Anderson makes the point that in the rehabilitation of the patient with thoracic disease a careful subclassification of the patient's disability must be the basis of treatment. He divides chest diseases into acute, long-acute, recurrent, relapsing, and chronic.

Dr. O'Malley gives a strong argument against diagnosing a patient as a neurotic in his chapter on rehabilitation in general and orthopedic surgery. He says this diagnosis is sometimes given patients because of the failure of the doctor to understand symptoms or to pay enough attention to them. "How ready we are, when puzzled or resentful, when the result of the treatment is not as good as it might be, to blame the patient; if the term neurosis is not on the tip of the tongue, it is the forefront of the mind." He also points out that sending a patient for resettlement or retraining is often just a sign of failure of direct rehabilitation.

The chapter on occupational therapy by Barbara Stow strongly argues that, if a hospital is going to have occupational therapy at all, it must have good occupational therapy with a well-equipped shop. It must also take the responsibility of seeing that there is good medical support for the occupational therapist.

In Doctor Ling's chapter on the organization of rehabilitation he shows that the hospital cannot advance in rehabilitation without the community's advancing at the same time. He also discusses some of the reasons why practicing physicians are slow to consider rehabilitation and yet they are absolutely necessary for the rehabilitative process if it is to have any continuity.

In reviewing this book, American readers may be unfamiliar with the role of a district rehabilitation officer in Great Britain. He is given much importance in the role of rehabilitation, and it may be wondered if there is a similar person in this country. The State Division of Vocational Rehabilitation is our equivalent and the Regional Vocational Counselor would be similar to the District Rehabilitation Officer.

I believe that there is a real gap in this book because rehabilitation nursing is not brought up

for comment. If rehabilitation is the new responsibility of hospitals, nursing will have an important part to play.

As a text to be used in teaching and as a text to stimulate doctors to think about rehabilitation, this book has real importance and has the added benefit of being extremely readable.

Harold N. Willard
Waterville, Maine

BOOKS RECEIVED

An Experiment in Mental Patient Rehabilitation. By Henry J. Meyer and Edgar F. Borgatta. Pp. 114, Russel Sage Foundation, New York, 1959. Price, $2.50.

A Practice of Thoracic Surgery. By A. L. d'Abreu. Pp. 619, The William & Wilkins Co., Baltimore, 1958. Price, $19.00.

Recent Advances in Respiratory Tuberculosis. By Frederick Heaf and N. Lloyd Rusby. Pp. 284. Little, Brown and Company, Boston, 1959. Price, $8.00.

La Corticotherapie Surrenale dans le Traitement de la Tuberculose. By Ch. Gernez-Rieux, J. Warembourg, and M. Pauchant. Pp. 216, Masson et Cie, Paris, 1958. Price, 2.600 fr.

Principles of Internal Medicine. Edited by T. R. Harrison, Raymond D. Adams, Ivan L. Bennet, Jr., William H. Resnik, George W. Thorn, and M. M. Wintrobe. Pp. 1839, McGraw-Hill Book Company, Inc., New York, 1958. Price, $18.50.

American Trudeau Society

Medical Section of the National Tuberculosis Association

ABSTRACTS OF MEDICAL PAPERS PRESENTED AT THE ANNUAL MEETING OF THE AMERICAN TRUDEAU SOCIETY HELD IN CONJUNCTION WITH THE MEETINGS OF THE NATIONAL TUBERCULOSIS ASSOCIATION AND THE NATIONAL CONFERENCE OF TUBERCULOSIS WORKERS, MAY 24–29, 1959

1A Clinical Tuberculosis

Patterns of Disease Associated with Atypical Mycobacteria in Texas

DANIEL E. JENKINS, DAVID BAHAR, IRVING CHOFNAS, RILEY FOSTER, HOWARD T. BARKLEY, FRANCES WHITCOMB, MARTHA FOSTER, ELIZABETH JONES, and HELEN MCGEE, Houston, Texas

Approximately 75 patients with clinical disease associated with the isolation of atypical mycobacteria are reported upon. The organisms fall into the following groups according to Runyon's latest classification: Group I (photochromogens) 62, Group II (scotochromogens) 4, Group III (Battey strain) 2, and Group IV (pleomorphic, branching) 7.

All patients except two had bronchopulmonary manifestations. One patient exhibited only lymph node disease from which photochromogens were isolated, and the other a perirectal abscess in which Group IV organisms were found. Chest roentgenograms allowed the following classification of extent of the disease process: minimal 8, moderately advanced 37, far advanced 27, hilar adenopathy 2, cavitary 50, and sizable pneumonic component 20.

Only patients with pure cultures of atypical mycobacteria are included, and those in whom *M. tuberculosis* or mixed strains were encountered have been omitted.

Certain interesting features of this group of patients appeared:

1. Since long periods of therapy and observation are available on most of the patients, an appraisal of results was made.

   a. Correlation between *in vitro* susceptibility studies, drugs employed in therapy, and clinical results tend to emphasize the importance of a proper choice of drugs and the avoidance of bacterial resistance to those drugs to which pretreatment susceptibility exists.

   b. Approximately 33 per cent of patients have undergone chest surgery, mostly resection, with very good results and a long period of postoperative follow-up (mean of 26 months). This differs from some previous reports in which surgical results have been associated with a high incidence of complications. There is a high incidence (70 per cent) of positive bacteriology in resected specimens.

   c. The disease of four patients has relapsed after many months of inactivity; and the same strain of organisms has been subsequently recovered.

2. All the patients listed in Runyon's Group IV harbor atypical mycobacteria which, after cultivation on laboratory media, exhibit branching. Other cultural characteristics are not similar to *Nocardia asteroides*. The patients have mostly chronic and cavitary lung pathology.
3. Limited data have been collected on the use of group specific tuberculin and gel diffusion antibody studies in these patients.

Chronic Pulmonary Tuberculosis in Individuals with Known Previous Primary Tuberculosis—An Analysis of 121 Cases

EDITH M. LINCOLN, LILIAN A. GILBERT, and SOLEDAD M. MORALES, New York, N. Y.

Between 1930 and 1956, 121 patients with chronic pulmonary tuberculosis about whom the records of a previous primary infection were available were seen in the Chest Clinic of the Children's Medical Service of Bellevue Hospital. The majority of these patients were first observed as children with primary pulmonary tuberculosis and then later developed evidence of disease while under observation for follow-up purposes. The remaining cases were first seen in the pediatric age range as cases of chronic pulmonary tuberculosis, but it was possible to get records of a previous primary infection. There were 83 girls and 38 boys in the group. Of the 83 survivors, 69 have been followed to the age of 21 years. When the first diagnosis of chronic pulmonary tuberculosis was made, 48 were between the ages of 6 and 12 years. Only 34, or about 28 per cent, received any form of antimicrobial therapy. In the entire group of 121 patients the interval between the first evidence of primary tuberculosis and chronic pulmonary tuberculosis varied from three months to 20 years. In almost one half of the girls chronic pulmonary tuberculosis was discovered within two years of

menarche. The mortality rate from tuberculosis was 23 per cent in the girls and 7 per cent in the boys. The disease was first diagnosed in a minimal stage in two thirds of the girls who later died of tuberculosis.

Corticosteroids in the Treatment of Pulmonary Tuberculosis

G. H. Hames, Lantana, Fla., Benjamin L. Brock, Orlando, Fla., Albert G. Lewis, Tampa, Fla., Lawrence C. Manni, Carl C. Birkelo, and Roberts J. Davies, Tallahassee, Fla.

The place of corticosteroids in the therapy of pulmonary tuberculosis has been the subject of much interest and discussion in the last few years. These hormones have been shown to be of value in counteracting hypersensitivity reactions to antituberculosis drugs and to alter favorably the course of the disease in patients who were critically ill with extensive disease and severe toxicity. Corticosteroids might enhance the results of treatment in patients with far advanced disease who are not considered critically ill.

In November, 1957, a study was initiated by the Florida State tuberculosis hospitals designed to explore the possible value of steroids as an adjunct to the routine chemotherapy of patients admitted with far advanced disease, excluding those who were considered critically ill. Newly admitted patients who had not had more than 30 days of chemotherapy were accepted for the study, provided that none of the usual contraindications to steroid therapy were evident. The patients were divided into groups on the basis of age, race, sex, and total extent of lung involvement. Alternate patients in each group received prednisolone for a minimum of four weeks, in addition to 300 mg. of isoniazid and 12 gm. of PAS daily, with which all patients in the study were treated.

The results in the two treatment groups are being evaluated on the basis of rate of clinical improvement, X-ray clearing, cavity closure, sputum conversion, and evidence of side effects attributed to the steroid therapy. Evaluation of the results of treatment in 132 patients at six months will form the basis for the proposed presentation. Of these 132 patients, 75 received prednisolone and 57 were in the control group.

The results observed in 100 patients at three months indicated significantly more rapid clinical and X-ray improvement in the prednisolone-treated patients, with no significant difference in the two groups regarding cavity closure or sputum conversion. No serious side effects due to prednisolone were encountered. It is hoped that evaluation of the larger group over a longer period of time will permit more definite conclusions as to the value and safety of steroid therapy in far advanced pulmonary disease.

The Impact of Corticosteroid Hormones in the Treatment of Tuberculous Meningitis

B. F. Voljavec, and Raymond F. Corpe, Rome, Ga.

The purpose of this paper is to compare the results of the treatment of tuberculous meningitis with antimicrobials alone and with corticosteroids added to a similar antimicrobial regimen.

All the patients included in this series are Negroes. The two therapeutic groups were formed by choice, not by randomization. The strict criteria used for selection enabled us to obtain two comparable groups for analysis.

The 33 patients received similar dosages of isoniazid, streptomycin, and PAS, except for one patient who did not receive PAS. Four patients had intrathecal streptomycin, and 16 patients received corticosteroid hormones.

The prognostic factors of marginal age, severity and duration of the disease, stage of the disease, cerebrospinal block, low value for C. S. F. sugar, severe pulmonary disease, and incidence of negative reaction to tuberculin were similarly distributed in both groups. Each unfavorable prognostic factor was assessed numerically. A numerical prognostic index was then determined for every case. Of particular interest is the fact that all four patients with block treated with antimicrobials alone died, and of the nine patients with block who received corticosteroids in addition to antimicrobials, only one died. A hypothesis is offered in regard to the effect of corticosteroids on the exudate that causes block in tuberculous meningitis.

In these two similar groups of Negro patients with tuberculous meningitis, only 19 per cent died when corticosteroids were added to the basic treatment regimen, as compared with 53 per cent who died without the benefit of corticosteroid therapy.

In conclusion, corticosteroids are a valuable adjunct in the treatment of tuberculous meningitis.

Relapse Experience of Optimally Treated Patients

Thomas F. Sheehy, Jr., Seattle, Wash.

Six hundred and sixty-nine tuberculous patients who were sputum negative at least six months prior to discharge in 1953 were followed for five years to identify factors related to relapse. The patients were selected from a total of 1,022 permanent dischargees in that year, and the selection was on the basis of criteria in vogue at that time. These criteria were sputum negativity for a six-

month period and all live dischargees; with these exceptions there was no further selection. Status was determined as of December 31 of each year. Relapse is defined as the occurrence of positive bacteriology on a single or repeated occasion, either by smear or culture or by X-ray worsening not explainable by some other acute identifiable cause.

By January 1, 1958, the disease in a total of 554, or 83 per cent, had not relapsed and the patients were still living. There were 42 deaths from nontuberculous causes and three deaths from tuberculosis in patients whose disease had relapsed. Relapse occurred in 11.2 per cent of the original sample; 9.6 per cent occurred in the first year. The follow-up status was determined as of January 1, 1959.

In conclusion, statistically it is shown that relapse occurs more commonly in middle-aged males with far advanced, cavitary disease with a secondary diagnosis of alcoholism who leave the hospital against advice. Conversely, in order to avoid relapse, it is more favorable to be a young female, to have noncavitary disease, and to undergo resection. Apparently, longer periods of chemotherapy prior to discharge and prior to final determination of status result in a favorable prognostic outlook.

Chemotherapy in the Surgical Treatment of "Salvage Cases" of Pulmonary Tuberculosis

MARJORIE M. PYLE, HIRAM T. LANGSTON, and KARL H. PFUETZE, Chicago, Ill.

The salvage cases in this study included 112 cases in which extensive resection was necessary and 109 cases involving problems of previous chemotherapy, bacterial resistance (particularly to streptomycin and isoniazid), and/or grossly positive sputum at the time of surgery.

In many cases the two groups overlapped. Of 75 patients undergoing pneumonectomy, more than a third had resistant strains and positive sputum before surgery.

Maximum protection was provided by complete evaluation of each patient and careful planning of his chemotherapy. In 97 cases, one or more of the minor antituberculosis agents were used—viomycin, pyrazinamide, and cycloserine (in high dosage). Intravenous PAS, isoniazid in large doses, and the inclusion of oxytetracycline in the patient's regimen were other expedients.

Both immediate and longer-term results among the "salvage cases" compared well with those obtained in cases of less extensive resection and/or more nearly optimal chemotherapeutic protection.

The Treatment of Tuberculosis with Alternating Antituberculous Drugs

E. T. PEER, St. Catharines, Ontario, Canada

Since tubercle bacilli have never been shown to develop resistance to a drug in less than three weeks when the drug is used alone, and appreciably longer than this when two drugs are used together, the effectiveness of drugs might be prolonged indefinitely by alternating them. This paper will present approximately two hundred primary-treatment cases treated in this way from April, 1954 to September, 1958. Most forms of tuberculosis (pulmonary, genitourinary, skeletal, lymphatic) were encountered and in varying degrees. Patients of all ages have been treated, from young adults to octogenarians.

The regimen most commonly used has been 1 gm. of streptomycin (or duo-streptomycin) at least three times weekly used with 12 gm. of PAS (or B-PAS) daily for the first month, and 400 mg. of isoniazid per day used with 3 gm. of pyrazinamide daily for the second month. After the second month, the regimen used during the first month was begun again. It will be seen that no drug was used for longer than one month at a time. When streptomycin and PAS were used first, streptomycin was frequently used on a daily basis for the first month. Cycloserine, oxytetracycline, etc., have been used as substitutes on occasion. The regimen has usually been continued for at least twelve months.

On this regimen every patient became bacteriologically negative six months after beginning treatment (most patients became negative after two or three months), and not a single patient has shown bacteriologic relapse to date.

There has been a notable reduction in toxicity on this regimen, particularly in the use of pyrazinamide. It has not been necessary to discontinue pyrazinamide because of laboratory or clinical evidence of liver disease.

1B Pulmonary Physiology

The Influence of Increased Intra-alveolar Pressure on the Uptake of Carbon Monoxide by the Lungs and on the Diffusing Capacity of the Pulmonary Membrane and Pulmonary Capillary Volume in Man

THEODORE C. SMITH, JOHN M. RAMLO, RICHARD A. COOPER, CARL B. WESTON, and JOHN RANKIN, Madison, Wisc.

The purpose of the present investigation was to determine the effect of an increase in intra-alveolar pressure on the uptake of carbon monoxide by the lungs (and hence on the calculation of diffusing

capacity), and by repeated measurements of overall diffusing capacity (D_L) at several different oxygen tensions to obtain estimates of the magnitude of change in the diffusing capacity of the pulmonary membrane and average pulmonary capillary blood volume.

Measurements of D_L were performed on healthy adult subjects by the carbon monoxide-helium breathholding technique at oxygen tensions between 100 and 600 mm. of mercury both at atmospheric pressure (open glottis) and at voluntarily maintained pressures (Valsalva maneuver) up to 80 mm. of mercury higher.

The results were as follows: (*1*) An average increase in intra-alveolar pressure of 60 mm. of mercury produced a calculated decrease in D_L of about 20 per cent. (*2*) There is a progressive decrease in the rate of carbon monoxide uptake during the period of breathholding at increased pressure, and D_L and average pulmonary capillary blood volume may decrease as much as 50 per cent after 10 seconds when there is a marked reduction in pulmonary capillary blood flow. (*3*) Indicator dye dilution curves revealed that little or no blood entered the thorax during the Valsalva maneuver. (*4*) Involuntary increases of intra-alveolar pressure of a magnitude found during ordinary clinical testing produce a measurable but clinically insignificant decrease in calculated D_L.

A Closed-Circuit Method of Determining Carbon Monoxide Diffusing Capacity of the Human Lung

Hollis G. Boren, and R. E. Collins, Houston, Texas

The closed-circuit method of determining the carbon monoxide diffusing capacity of the human lung provides a means of measuring simultaneously diffusing capacity, functional residual capacity, and mixing efficiency. It allows variation of concentration of inspired oxygen and may be used following exercise.

A constant-volume, closed-circuit system is used with continual recording of helium and carbon monoxide concentrations. New equations for calculating diffusing capacity are derived. The continuous variation in concentration of carbon monoxide in inspired and expired air is taken into account. Tidal volume, frequency of breathing, and functional residual capacities are included as parameters.

Results obtained to date have shown that the diffusing capacity of the lung varies with exercise and exhibits a limiting maximal value. The method is clinically applicable so that the frequency of diffusional defects may be studied. It does not require blood samples and provides excellent resolution of normal persons from those with diffusional defects.

The Simultaneous Relationships of Diffusing Capacity and Pulmonary Capillary Blood Flow to Alveolar Volume from Areas of the Lung Differing in Ventilation in Normals and Patients with Emphysema

William S. Spicer, Jr., Baltimore, Md., Robert L. Johnson, Jr., Dallas, Texas, and Robert E. Forster, Philadelphia, Pa.

Simple modifications in the bag-box spirometer system used to measure diffusing capacity (D_L) and pulmonary capillary blood flow ($\dot{Q}_c$) make it possible to measure these factors simultaneously from areas of the lung differing in ventilation. Nineteen determinations in four normal subjects resulted in mean values for D_L and $\dot{Q}_c$ from well-ventilated areas of 26.1 ml./min./mm. Hg. and 5.97 L./min., respectively. From the poorly ventilated areas the mean values were D_L, 26.1 ml./min./mm. Hg. and $\dot{Q}_c$, 5.51 L./min. Determination in seven patients with emphysema gave mean values of well-ventilated D_L, 21.2 ml./min./mm. Hg. and $\dot{Q}_c$, 4.50 L./min. and poorly ventilated (15.4 per cent difference in helium dilution) D_L, 21.9 ml./min./mm. Hg. and $\dot{Q}_c$, 5.05 L./min. However, there was considerable variation among the patients with emphysema, and in one patient complete absence of diffusion and blood flow was found in the poorly ventilated areas. In addition, an artefact that may affect the measurements in normals and markedly affect those in patients with emphysema (up to 50 per cent error) was demonstrated. The physiologic cause of this artefact was also demonstrated.

In the individuals tested:

1. D/V_A (ratio of diffusion capacity to total alveolar gas volume) was distributed independently of ventilation.

2. An artefact in the standard method was demonstrated, and a means of circumventing this was offered.

3. There was the suggestion that diffusing capacity is not necessarily distributed proportionally to pulmonary capillary blood flow.

4. The uniqueness of the method was demonstrated by measuring simultaneously the presence and the absence of perfusion in various areas of the lung of a patient with cystic changes.

Dimensional Responses of Bronchi to Airway Pressure, Gases, and Drugs

Kaye H. Kilburn, Denver, Colo.

The changes in length and diameter of bronchial segments in response to various stimuli were

visualized in 14 lightly anesthetized dogs by instilling Dionosil® into the right main bronchus. With the dog's position fixed and respirations arrested by succinylcholine, lateral bronchograms were made at endobronchial pressures of 5, 10, and 15 cm. of water. Films to assess gas and drug responses were obtained at 10 cm. of water.

Five cm. of water airway pressure altered lengths of lobar bronchi −7 to +9 per cent and increased diameter 12 to 63 per cent; 10 cm. of water increased lengths 2 to 23 per cent and diameters 23 to 93 per cent; 15 cm. of water slightly augmented diameters; 5 cm. of water maximally distended subsegmental and segmental bronchi.

Intravenous acetylcholine constricted subsegmental bronchi to fine lines and produced knobs in larger bronchi. Lengths were reduced 5 to 10 per cent, and diameters 16 to 62 per cent. Acetylcholine by aerosol constricted main and diaphragmatic bronchi without affecting smaller bronchi. This was due probably to distribution of particles.

Histamine constricted distal, subsegmental bronchi while all proximal bronchi changed less; diameters were reduced 8 to 42 per cent and lengths 0 to 22 per cent.

Serotonin produced extreme beady constrictions of small bronchi and narrowed lobar bronchi and the trachea. Lobar bronchial lengths were reduced 0 to 18 per cent and diameters narrowed 27 to 76 per cent.

Isoproterenol following acetylcholine, histamine, or serotonin restored pretreatment dimensions. Diameters approximated, but length exceeded, controls 5 to 15 per cent. Atropine restored diameter (−11 to +13 per cent) but not length and failed to block acetylcholine constriction.

Breathing 10 per cent carbon dioxide for 3 to 10 minutes reduced bronchial diameters 5 to 60 per cent and lengths 3 to 12 per cent without producing distortion, and results of 100 per cent oxygen breathing were inconclusive.

A neglected method for studying bronchial physiology demonstrated greater alterations of diameter than length in response to most stimuli. Bronchial spasm produced by acetylcholine, histamine, and serotonin is blocked by isoproterenol. Atropine is less effective.

Differential bronchography might aid the early recognition of diseases that alter bronchial elasticity.

Cardiopulmonary Function in Chronic Obstructive Emphysema

M. Henry Williams, Jr., and Lenore R. Zohman, Valhalla, N. Y.

Data have been obtained on various aspects of cardiorespiratory function in 22 cases of chronic pulmonary emphysema. Each patient had repeated measurements of maximal midexpiratory flow rate (MMF) and vital capacity, of arterial blood gases during rest and exercise, of diffusing capacity by the steady-state carbon monoxide method during rest and exercise, and, less frequently, of pulmonary vascular pressures and flows during rest and exercise. From these data, the pathophysiology of chronic pulmonary emphysema emerges as a fairly homogenous picture of chronic, severe airway obstruction (MMF never greater than 1 liter per second in over 200 measurements), mild hypoxemia and carbon dioxide retention only slightly increased by exercise, pulmonary hypertension particularly during exercise, severe reduction of the diffusing capacity during rest and exercise (never greater than 12 cc. per minute per mm. of mercury during exercise), and a normal resting cardiac output that was slightly subnormal during exercise. These abnormalities were examined in terms of their pathogenesis and their relationships to the structural abnormalities present in pulmonary emphysema. Particular attention was devoted to the contributions of reduced alveolar ventilation, impaired diffusion, right-to-left shunts, and disordered ventilation/perfusion relationships in the lung to the development of hypoxemia and exercise intolerance. Histories of selected patients followed over a period of three years with repeated measurements of all functions illustrate the fact that the functional abnormalities in pulmonary emphysema need not become progressively more severe.

The Syndrome of Pulmonary "Hyperinflation": An Early Stage of Chronic Pulmonary Insufficiency—A Preliminary Report of 40 Cases

James C. Syner, and Joel H. Richert, Denver, Colo.

Pulmonary function studies were carried out in patients undergoing medical chest evaluation. In 40 of these patients, each of whom had a normal ventilatory profile as judged by measurements of external spirometrics, significant abnormalities were detected in lung volume measurements and lung compartment relationships. The abnormalities consisted of increased size of the functional residual capacity, residual volume, and total lung capacity and elevation of the residual volume total lung capacity ratio and mixing index (per cent of alveolar nitrogen).

These changes are compatible with those observed in obstructive pulmonary emphysema. The striking differences between this state of "hyperinflation" and obstructive emphysema are: (*1*) normal external ventilation and (*2*) absence of airway obstruction. In selected cases associated

abnormalities were detected and included: hypoxia, hyperventilation or treadmill exercising, and decreased oxygen diffusing capacity.

Clinical findings usually consisted of cough, sputum production, frequent and persisting respiratory infections, chest pain, exertional dyspnea, coarse rhonchi, and a normal chest roentgenogram. Chronic bronchitis was the usual clinical diagnosis.

This state will be missed if a normal ventilatory profile is accepted as evidence of normal pulmonary function. It is considered to represent an early stage of chronic pulmonary insufficiency.

The Roentgenographic Determination of the Total Lung Capacity in Health, Emphysema, and Congestive Heart Failure

JOHN A. PIERCE, HOWARD J. BARNHARD, JOHN JOYCE, and JOSEPH BATES, Little Rock, Ark.

A simple method for the determination of total lung capacity could be useful in clinical medicine. A new technique has been developed that considers the shape of the lungs as elliptical in transverse or cross section. Corrections are measured for the volume of the heart and the space occupied by the diaphragmatic domes. A calculated quantity is deducted for the intrapulmonary blood and tissue volumes.

The roentgenographic method has been compared to conventional spirometric-nitrogen dilution measurements of total lung capacity in 74 subjects. These subjects were in four groups as follows: healthy young, 21, healthy old, 25; emphysema patients, 16; and patients with congestive heart failure, 12.

A high degree of correlation was found between the two methods of measuring total lung capacity in the healthy young group ($r = 0.88$), the healthy old group ($r = 0.88$), and the group with congestive heart failure ($r = 0.87$). A significantly lower coefficient of correlation was obtained in the emphysema group ($r = 0.41$). The mean total lung capacity in these patients was 0.8 liters more when measured roentgenographically than when measured with the spirometer dilution technique. This finding can be attributed to errors in the nitrogen dilution method of measuring the residual volume in patients with emphysema. In the literature, pressure volume diagrams of the lung in emphysema are in error wherever the volume has been determined with the nitrogen dilution method. The volume elastic properties of the lungs are more severely altered in patients with emphysema than was previously indicated.

These findings agree with the results of Bedell, Marshall, DuBois, and Comroe, who studied intrathoracic gas volumes with a body plethysmograph. The present roentgenographic technique was also compared with previous roentgenographic methods and further studied in a small series of the cases reported by Bedell and associates. The method appears to be sufficiently accurate for clinical use.

The Estimation of Ventilatory Function by Means of Fluoroscopy

GORDON L. SNIDER, and ALLAN SHAW, Chicago, Ill.

Fluoroscopy of the chest has long been advocated as an important tool for the evaluation of ventilatory function. In order to evaluate the accuracy of prediction of ventilatory function from fluoroscopy, estimates of the maximum breathing capacity (expressed as per cent predicted normal) were made at the end of routine fluoroscopic examination on all patients studied in the pulmonary function laboratory. The fluoroscopist had no prior knowledge of the patients.

Two groups of 100 patients were then collected for analysis. These were all patients with pulmonary tuberculosis who had been referred to the laboratory for evaluation of risk prior to thoracic surgery for treatment of their disease. The cases were unselected except that they represented consecutive fluoroscopic examinations by one of the writers. These were performed on one of the groups between October, 1953 and February, 1955 and on the other group between April, 1957, and March, 1958.

The difference between the maximum breathing capacity as predicted fluoroscopically and the laboratory measurement of maximum breathing capacity was designated the error of fluoroscopic estimate. The mean fluoroscopic error for the 200 cases was 11.4 per cent of predicted normal with a range of ± 39 per cent normal and a standard deviation of 9.6 per cent normal. In 83 cases ventilatory function was overestimated and in 88 it was underestimated. Fluoroscopic estimate coincided exactly with determined data 29 times; 53 per cent of the cases fell in the range of ± 10 per cent normal; however, 20.5 per cent of the estimates were found to be beyond the range of error of ± 20 per cent normal. Comparison of the error of fluoroscopic estimates in the two groups showed there was an effect of experience on the accuracy of the fluoroscopist. Thus, the mean fluoroscopic error fell from 13.1 per cent normal in the first group to 9.3 per cent normal in the second group, and this difference was statistically significant.

A technique was also devised for estimating ventilatory function from a full inspiration chest film that was taken during the period of laboratory study. The mean error of estimate of ventilatory function based on the X-ray film was 17.1 per cent of normal. This was significantly larger than the

error of fluoroscopic examination. The error of roentgenographic estimate was consistently in the direction of underestimating function.

Other factors possibly affecting the accuracy of fluoroscopic estimate were also investigated, such as the presence or absence of mediastinal shift, the presence of previous surgery, the presence of airway obstruction, the severity of measured ventilatory function abnormality on the amount of roentgenographic abnormality.

It is concluded that, although fluoroscopy remains a very valuable tool in estimating ventilatory function, the error of estimate is very large in about 20 per cent of patients. Fluoroscopy cannot be used when precise measurements are necessary.

Pulmonary Vascular Resistance in Acquired Heart Disease

Bernard L. Charms and Bernard L. Brofman, Cleveland, Ohio

The role of the pulmonary vasculature in congenital and acquired heart disease has received considerable attention. Pathologically, medical hypertrophy and intimal proliferation have been demonstrated. It is generally accepted that these changes develop to protect the pulmonary capillary bed against high flows or pressures.

Temporary unilateral pulmonary artery occlusion has been demonstrated as a useful tool and a unique method for increasing pulmonary blood flow without over-all change in cardiac output or left atrial pressure such as occurs with exercise. Accordingly, seventeen patients with acquired heart disease were studied, both before and after sudden occlusion of either right or left main pulmonary artery by an inflatable cuff on the middle lumen of a special triple lumen cardiac catheter. Proximal and distal (or wedge) pressures were measured, cardiac contents determined, and pulmonary arteriolar resistance calculated before and after occlusion. All patients had elevated wedge or distal pressures ranging from a mean of 10 to 35 mm. of mercury, and pulmonary artery pressures ranged from means of 26 to 90 mm. of mercury. With occlusion, in all but one case, there was no change in pulmonary artery pressure. Since total cardiac output was perfusing a single lung, that lung, irrespective of initial resistance, was able to accommodate the abrupt increase in flow without pressure change. Resistance was, therefore, abruptly lowered to approximately one half the control value.

It is concluded that, despite the anatomic evidence of pulmonary arteriolar change, the pulmonary vascular bed can accommodate an increase in flow at a greatly reduced resistance. It is suggested that extravascular factors, such as increased intra-alveolar pressure secondary to bronchoconstriction, may play an important part in the pulmonary resistance and pulmonary hypertension seen in acquired heart disease.

2A Symposium on Pulmonary Emphysema

The Elastic Fiber System of the Human Lung

Clayton G. Loosli, Los Angeles, Calif.

(Abstract not available at time of publication)

Pathology

David M. Spain, Brooklyn, N. Y.

(Abstract not available at time of publication)

Relationship Between Bronchitis and Emphysema

Charles M. Fletcher, London, England

(Abstract not available at time of publication)

The Pathology and Pathogenesis of Emphysema

Kenneth H. McLean, Rochester, Minn.

The pathogenesis of emphysema is not simple. It has been suggested that emphysema results from the effects of a single process, such as mechanical stress, ischemic atrophy, or an undefined type of atrophy, but such hypotheses are unsatisfactory. The general hypothesis that the disease is a sequel of inflammatory damage to the small airways is considerably more attractive. One shortcoming of this hypothesis is that the natural history of bronchiolitis has not been adequately studied. Study of bronchiolar inflammation in its commoner forms indicates that complete obstruction of small bronchioles and respiratory bronchioles is an integral part of the process. In simple acute bronchiolitis, the aeration of the more distal passages is maintained by collateral ventilation. When bronchioles are obstructed by plugs of mucus or exudate, bacteria in the plug may proliferate and cause super-added bacterial bronchiolitis if the plug is retained for a sufficient period.

Permanent structural changes in the bronchioles are not uncommon after any form of bronchiolitis. These changes include partial or complete obliteration of the lumen and damage to wall structures, but their recognition may be masked by reparative processes. Tissues representing an obliterative bronchiole tend to become less conspicuous, whereas any air passage lined with connected tissue, regardless of whether it is a damaged bronchiole or a new pathway, tends to develop the structure approximating that of a normal bronchiole. The difficulty with which some old inflammatory damage is recognized readily leads to underestimation of the extent of such damage.

If lesions are sought in which there is minimal emphysema, enough of the original structure can be recognized for the observation to be made that emphysematous change occurs in the immediate vicinity of air passages in which evidence of old inflammatory damage can be identified. This association was seen most clearly in the form of the disease that has been termed "centrilobular" emphysema but was also recognized readily in small localized emphysematous lesions.

An hypothesis based on these observations of the natural history of bronchiolitis may be outlined as follows. During an attack of acute bronchiolitis there is diffuse obstruction of small bronchioles by mucus and exudate. The air passages beyond the occluded bronchioles are ventilated by collateral ventilation and, although this ventilation is sufficiently good to prevent arterial desaturation, air enters and leaves these regions more slowly than it does in a normally ventilated lung. With a violent expiration, such as a cough, a normal lung is subject to little or no stress; but where diffuse bronchiolar obstruction exists, considerable local pressure gradients persist for an appreciable time and exert unusual stresses upon the tissues in the area. If the plugs are not properly expelled, this stress will be repetitive and will only cease when either the plug is expelled or sufficient breakdown of tissue occurs to permit freer collateral ventilation. A normal lung probably withstands this stress relatively well, but tissue, the elasticity of which is reduced by inflammation, tends to stretch and break with repeated stress. The application of this hypothesis may be seen most clearly in minimal centrilobular lesions in which the air passages involved in the emphysematous process are those immediately related to the region of bronchiolar inflammation.

The progress of the disease would then depend upon the duration, severity, and number of subsequent inflammatory episodes. Repeated minimal inflammatory episodes might lead to slow discontinuous progress of the disease, but an equivalent amount of damage could result from one severe prolonged episode. With progress, dilatation and breakdown of the tissues comprising the finer airways involves the whole lobule, interlobular septa, and finally even intersegmental septa. Tension of surrounding structures on the bronchiolar wall decreases and bronchioles collapse more and more readily on expiration, aggravating the trapping of air in the emphysematous regions and so accelerating the rate of progress of the disease.

Inflammatory processes leading to emphysema range from subclinical pneumonitis through the more common respiratory infections such as influenza and bronchitis to the more dramatic illnesses of sufficient severity to be recalled by the patient after many years when questioned by his physician. It would be expected, therefore, that pathologic evidence of past inflammatory processes frequently occurs in the absence of memorable respiratory illnesses.

Localization of Pulmonary Emphysema

DAVID V. BATES, Montreal Canada

(Abstract not available at time of publication)

Basic Physiologic Abnormality

GILES F. FILLEY, Denver, Colo.

(Abstract not available at time of publication)

2B Differentiation of Mycobacteria

Atypical Acid-fast Bacilli

SHIRLEY McMILLEN, and DANIEL S. KUSHNER, Chicago, Ill.

Ten per cent of all acid-fast bacilli isolated from the more than 30,000 specimens submitted to the tuberculosis laboratory of Cook County Hospital over a five-year period (1954–1959) were not typical of *M. tuberculosis*.

The atypical organisms isolated over this five-year period included only 42 strains of rapidly growing acid-fast bacilli, which were identified as *M. fortuitum*, 28; *M. smegmatis* 7; *M. phlei* 2; *M. rhodochrous*, 5. The rest of the atypical isolates, all slowly growing organisms, separated into three groups based upon pigment production: nonchromogenic mucoid growth, photochromogenic dry growth, and scotochromogenic mucoid growth.

Seventy-eight strains of the slowly growing atypical isolates have been investigated, employing type cultures of *M. bovis*, *M. intracellularis*, *M. kansasii*, *M. marianum*, *M. scrofulaceum*, and *M. paraffinicum* for a comparative study of their cultural and biochemical behavior. Determinations included characteristics of growth on Löwenstein-Jensen, glycerol, blood, EMB, and cornmeal solid media, in Penassay and Dubos broth media, and the use of organic acid salts.

To date the data indicate (*1*) the prevalence of atypical strains in unselected cultures and the predominance of the slowly growing types, (*2*) the occurrence of three distinct groups of the slowly growing organisms, and (*3*) the definition of some of the characteristics of these groups in comparison with named Mycobacterium species.

An Attempt to Subgroup the Acid-fast Bacilli on the Basis of Their Catalase Activities: Preliminary Report

GEORGE P. KUBICA and GLEASON POOL, Chamblee, Ga.

It is well known that mycobacteria produce the enzyme catalase. But strains of acid-fast bacilli

that become resistant to isoniazid frequently lose, or experience a lowering of, their catalase activity. This loss of catalase activity, which seems to accompany increased microbial resistance to isoniazid, also has been correlated with attenuation in virulence of said microorganisms for the guinea pig. The suggested correlation between catalase activity and virulence of mycobacteria led Schweiger *et al.*, to develop an experimental procedure for distinguishing virulent mycobacteria from avirulent and saprophytic strains. The purpose of this study was to simplify the procedure of Schweiger and to extend the investigation to include other varieties of acid-fast bacilli likely to be associated with human tuberculosis or with clinical materials isolated from suspected tuberculous patients.

Studies were performed on 60 cultures of various types of acid-fast bacilli, including representatives of the following groups: human, bovine, avian, avirulent, saprophytic, cold-blooded, drug-resistant, and anonymous (atypical) acid-fast bacilli. The first studies were done on cultures obtained from egg slants; however, it was found that the test also could be performed on cultures grown in Tween®-albumin broth. Catalase tests were performed either in buffered solutions (pH 1, 7, 10) or in broth suspensions at room temperature (approximately 25°C.), or at 56°C., or at 68°C. Except in cases in which specific pH was desired, it was found that buffered suspensions were not needed and that cells could be left either in the original broth suspensions or on culture slants. For a standard test, culture suspensions were left in the respective pH-buffered solution or controlled-temperature water baths for twenty minutes. To the bacterial suspension was then added 1 ml. of a 1:1 mixture of Tween 80 and superoxol. Catalase reactions were given arbitrary values from 1 to 5+, depending upon speed of reaction and extent of bubbling.

Most cultures were catalase positive at room temperature and pH 7; human, bovine, and avian varieties of *M. tuberculosis*, however, reacted very slowly under these conditions, and on occasion it was impossible to detect any evolution of bubbles from these strains. Altering the pH from 1 to 10 did not seem to produce either a consistent or a reliable change in the catalase activity of the various groups. By elevating the temperature of the culture suspension to 68°C., human, bovine, and avian varieties of *M. tuberculosis*, regardless of virulence, lost their catalase activity; most saprophytes and Groups I, II, and IV of the anonymous strains retained their catalase activity at this temperature, and Group III strains exhibited variable results under these conditions. Results were tabulated for comparison.

Although this test in itself cannot be used for typing purposes, its use in conjunction with other cytochemical tests might prove useful in identifying or grouping the acid-fast bacilli. Further studies along this line are under way.

A New, Rapid Concentration and Differential Culture Technique for the Mycobacteria and Nocardia Organisms

JAMES B. GRAY, Jamaica Plain, Mass.,
EDITH REINISH, Westfield, Mass.,
and SANFORD CHODOSH,
Boston, Mass.

A rapid, five-day method for the concentration and differential culture of acid-fast organisms has been developed. The concentration technique utilizes a new muco-polysaccharide digesting agent. During this process of digestion, the acid-fast organisms selectively adhere to a specifically coated cover slip while in a roller drum. After five hours, the cover slip is placed in Dubos liquid serum medium for five days. The cover slip is then stained and mounted. The acid-fast microcolonies are then examined. The virulent, avirulent, and nocardia types can be identified by their distinctive growth characteristics.

The results of a trial of this method have been compared with the routine culture method.

Preliminary Studies on the Identification of Atypical Nocardia Species through Application of Bacteriophage

HELEN J. SHADOMY and O. A. PLUNKETT,
Los Angeles, Calif.

In view of the increasing appearance of "atypical" acid-fast bacilli in pulmonary infections resembling clinical tuberculosis, an attempt was made to develop a system of bacteriophage typing which would permit the discrimination between true mycobacteria and true nocardia species.

Using modifications of standard enrichment techniques, pools of selected atypical nocardia species were prepared and enriched with broth and fertile garden soil. Following a preliminary incubation period, the pools were filtered and assayed for bacteriophage activity.

Of the bacteriophages or nocardiophages isolated by this method, 16 were selected for further study. These 16 nocardiophages were found to exhibit definite patterns of activity when tested on 176 strains of true nocardia species and no activity against 45 strains of mycobacteria and 60 strains of streptomyces. The nocardiophages were further found to be absolutely specific for only nocardia and could be associated with one or more typical nocardia species. However, numerous

instances were noted in which the sensitivity of the strains did not agree with their speciation.

The Intracutaneous Inoculation of Guinea Pigs as a Means of Determining the Virulence of Drug-resistant and Anonymous (Atypical) Acid-fast Bacilli: Preliminary Report

GEORGE P. KUBICA, R. EDWARD BEAM, ANNIE L. VESTAL, and GLEASON POOL, Chamblee, Ga.

With the increased isolation of drug-resistant and anonymous acid-fast bacilli, the value of the guinea pig test for virulence determination of acid-fast bacilli has markedly decreased, as so many of the above mentioned microorganisms show little or no virulence for guinea pigs. Subcutaneous, intraperitoneal, and even intracardial inoculation of large doses of these bacterial suspensions into guinea pigs elicits very little microscopic evidence of tuberculous involvement. There is, therefore, a need for a reliable virulence test for the troublesome groups of drug-resistant and anonymous acid-fast bacilli.

It was thought that perhaps the intracutaneous inoculation of guinea pigs, a technique that proved useful in distinguishing saprophytic from virulent mycobacteria, might provide a rapid, reliable means of determining the virulence of these questionable cultures. Accordingly, culture suspensions of one or two representatives of the various groups of acid-fast bacilli were prepared in graded doses and inoculated intracutaneously into guinea pigs. Representatives were obtained from the following groups: *M. tuberculosis* var. *hominis*, var. *bovis*, var. *avium*, several saprophytic mycobacteria, various anonymous acid-fast bacilli, and several drug-resistant mycobacteria. The final concentrations of cells inoculated intracutaneously into the animals were 0.1 mg., 0.01 mg., and 0.001 mg. The pathology observed ran the gamut from no visible lesions to an open, draining sinus. Preliminary success with this route of inoculation led to the inoculation into guinea pigs of suspensions prepared from a total of 41 stock or pedigreed strains.

As expected, all the human, bovine, and avian varieties of *M. tuberculosis* produced open, draining lesions. Two of three of the nocardia cultures caused open, draining lesions. Only one of six saprophytic strains caused any visible pathologic change; this one "saprophyte" is being studied further to see if perhaps it may be improperly named. In order of virulence for man, the anonymous acid-fast bacilli are generally listed as Groups I, III, IV, II, with the Group II (scotochromes) often classed as saprophytic, or at least of relatively low virulence for man. Following intracutaneous inoculation of guinea pigs, all the anonymous strains, except the scotochromes, produce open, draining abscesses. Most of the drug-resistant strains caused open lesions in the guinea pig.

From all outward appearances, the intracutaneous route of inoculation appeared to provide a better correlation with cultural virulence for man than did the usual subcutaneous and intraperitoneal routes. Following this preliminary study, the intracutaneous route of inoculation was included as a routine animal test for all reference cultures submitted to this laboratory. Although it is realized that these present conclusions are based on relatively meager data as far as numbers are concerned, the results are very promising. Further studies are under way to corroborate these preliminary data.

A Comparative Study of M. Avium and the Battey Type of Anonymous Acid-fast Bacillus

SEYMOUR FROMAN, LOIS SCAMMON, EMIL BOGEN, and J. P. MYLES BLACK, Olive View, Calif.

The Battey type of anonymous acid-fast bacillus produces disease in man indistinguishable from true pulmonary tuberculosis. A comparative study was therefore made of the Battey type and *M. avium*, which it closely resembles, in regard to (*1*) cultural, biochemical, and antigenic relationships, (*2*) virulence for the fowl, and (*3*) phage susceptibility, in order to determine the existence of any differential characteristics. A total of six strains of *M. avium* and nine strains of the Battey type were employed in these studies.

No clearly distinguishing characteristics were noted except that a small inoculum (0.01 mg.) of virulent *M. avium* uniformly produced progressive disease in the fowl, whereas a similar inoculum of the Battey type failed to produce any macroscopic evidence of disease. It was also noted that large inocula (approximately 5 mg.) of four of the nine Battey types examined produced lesions in the fowl compatible with that produced by *M. avium*.

Only two of the Battey strains were phage susceptible, indicating a distinct difference among strains of a group that otherwise appears homogeneous. None of the avian strains was phage susceptible.

On the basis of the data collected, it appears that the Battey type represents a variant of *M. avium*.

Observations on Nonculturable Acid-fast Bacilli in Resected Pulmonary Lesions

LAWRENCE G. WAYNE, San Fernando, Calif.

The significance of acid-fast bacilli, which are seen in a considerable proportion of resected

human pulmonary lesions but which cannot be cultured, has not yet been clearly established. This report presents (*1*) the results of attempted revival of such tubercle bacilli, (*2*) observations on the persistence of these bodies on prolonged anaerobic incubation in original tissue homogenates, and (*3*) observations on the autolytic processes of tubercle bacilli.

Attempts at revival of "nonviable" tubercle bacilli included the following: techniques for washout or adsorption of toxic substances in tissue homogenates; enriched culture methods; modifications of gaseous environment; prolonged incubation; animal inoculations by special routes and treatments. Of fifteen special techniques investigated, none could be demonstrated to result in revival of the nonviable organisms.

Aliquots of tissue homogenates with high counts of acid-fast organisms were also incubated under sterile mineral oil for periods of up to two years. Such treatment resulted in rapid disappearance of most of the acid-fast bodies in a given specimen. This was followed by a stabilization of the numbers of these bodies, which then persisted for two years or more. In *in vitro* culture studies, autolysis of tubercle bacilli, induced by nutrient deficiencies or by drugs, appeared to be an adaptive process, and old cultures of low reproductive activity showed a reduced ability to adapt to autolysis.

It is concluded that disappearance of acid-fast bacilli from tuberculous lesions is partially a function of autolysis and partially of dynamic host action on these bodies. In a situation in which host mechanisms fail to eliminate the nonviable bacilli, the autolytic mechanism often fails to dispose of all bacillary bodies, and visible but nonviable acid-fast bacilli may persist for long periods.

Nonacid-fast or Chromophobic Microorganisms in the Tuberculous Lesions of the Human Lung

WALENTY NYKA, Baltimore, Md.

Sixty-one human lungs, 58 of them surgically removed and three of them from autopsy cases, have been examined by means of a newly devised histologic technique. Of this number, 37 showed only cavitary lesions, and in 24 there were cavitary lesions in association with caseous nodules. In all these lesions, organisms have been found which appear in the form of individual granules, coccobacilli, or granular rods of varying length, occurring either singly or in clusters. They were scattered irregularly throughout the caseous material, and in cavities they were frequently mixed with acid-fast bacilli or located inside the leukocytes lining their walls. It is worth emphasizing that they were present in the nodular lesions of sputum-negative patients in which no acid-fast bacilli have been found, and that they were particularly numerous in softening caseum. These organisms do not stain with carbolfuchsin and show an exceedingly low affinity for other aniline stains, for which reason they have been called "chromophobic organisms." Of particular interest are the chromophobic organisms mixed with acid-fast bacilli because in such instances bacteria can be seen that are morphologically completely different from the acid-fast bacilli but are weakly acid fast, a finding which suggests that such cells may be in a state of transition between the acid-fast and nonacid-fast forms of the tubercle bacillus.

It is worth mentioning that chromophobic organisms, similar to those described in human lungs, have also been found in the organs of guinea pigs infected with human tubercle bacilli by the air-borne route and that recently they have been produced *in vitro*.

It is believed that the research so far conducted warrants the following conclusions:

1. By means of a special histologic technique, the presence of granular bodies can be demonstrated in the caseous lesions of the lung that are not revealed by the standard staining techniques.

2. There is histologic and experimental evidence to suggest that these organisms represent a variant form of acid-fast tubercle bacilli.

3. The great numbers of these organisms in the necrotic lesions of pulmonary tuberculosis suggest that they play an important role in the pathology of the disease.

4. The relationship among the chromophobic organisms, the Much granules, and the granular forms of tubercle bacilli described by other authors remains at the present time an open question that needs further investigation.

2C Tuberculosis Epidemiology

Finding Tuberculosis through Family Contact Investigation at a Children's Tuberculosis Clinic

KATHERINE H. K. HSU, Houston, Texas

Since the opening of the Houston Children's Tuberculosis Clinic in 1954, contact examination has been carried out on a limited scale. In July, 1957, with research grants obtained from the national, state, and local tuberculosis associations, a Family Contact Investigation Service was formally established at this clinic to coordinate a community-wide contact examination project through the cooperation of the existing facilities in Houston and Harris County.

The scheme of contact investigation differs from other case-finding projects in that thorough and repeated examinations are given to find in-

fected children, and young infected children are taken as a clue to locate the "nests" of tuberculosis in the community. Complete contact investigation is carried out only on children having a diagnosis of active primary tuberculosis. The immediate family as well as the associating families are examined for tuberculosis.

In the year 1957–1958, 825 child contacts of 129 children were investigated. Among the 825 contacts, 158 children were found to have active primary tuberculosis, 20 had cultures positive for *M. tuberculosis*, 66 showed X-ray evidence of tuberculosis, and 32 converted their tuberculin reactions within one to five months; 31 were under 36 months of age. None of these children had shown any sign of ill health that would have prompted their parents to seek medical examinations for them. Without contact investigation, practically all of these cases would have been missed.

Intensifying contact investigation and giving proper attention to childhood tuberculosis will greatly enhance tuberculosis control in the United States today. We have demonstrated that this can be carried out successfully with an organized effort of the local health agencies and proper education of the patients' families. Last year we were able to obtain reports on the examinations of 92 per cent of the 757 household contacts and 67 per cent of the 1,053 nonhousehold contacts.

The annual operating expenses for the Family Contact Investigation Service at the Houston Children's Tuberculosis Clinic amounted to $8,650.

Screening Procedures for Pulmonary Disease in Pregnant Patients: A Preliminary Report

Dorothea D. Glass, Fruma W. Ginsburgh, Katharine R. Boucot, Bernard Broad, Marie Capitanio, and Lenora Gray, Philadelphia, Pa.

Routine photofluorograms have been part of the prenatal program at Woman's College Hospital since 1944. Because of the publicity about radiation hazards, the Department of Preventive Medicine, early in 1957, suggested a change to tuberculin testing, with X-rays limited to reactors. However, the Department of Obstetrics was unwilling to adopt this policy because significant nontuberculous pulmonary disease had been discovered by X-ray that might have been overlooked if routine chest photofluorograms had been abandoned in nonreactors.

Over the six-month period from June 1 to December 31, 1957, arrangements were made to do both tuberculin tests and photofluorograms routinely on prenatal clinic patients at Woman's College Hospital. Of 308 women, one failed to report for her miniature chest film and three failed to report for skin-testing. Of the first 150 women tested with first-strength PPD, 49 per cent failed to return for the skin test reading. When this situation was recognized, a special effort was made to get the women to report back. Some tests had to be given two or three times. Finally, 13 per cent of the 308 women tested failed to complete their tests.

No significant photofluorographic lesion was found among tuberculin reactors or nonreactors, although 20 per cent had reacted to first-strength PPD and 13 per cent to both first and intermediate strengths. This finding was attributed to the fact that, since the hospital's fiscal policy called for a payment of $90, these women were at a relatively high socio-economic level for clinic patients.

Therefore, the study was extended to include 630 obstetrical patients hospitalized at a large city hospital, Philadelphia General Hospital, Blockley Division. These women were tested on admission, most often immediately post partum, over a six-week period between June 16 and September 1, 1958. Since this group of Blockley patients is composed largely of women on public assistance, it was thought that their lower socio-economic level would be associated with a higher percentage of tuberculin reactors and some clinical tuberculosis. The age and race composition of the two hospital populations was similar; 90 per cent were Negroes between 15 and 35 years of age. Interestingly enough, of the 432 women whose tests were read, 23 per cent reacted to first-strength PPD at Philadelphia General Hospital. Thirty-one per cent were discharged before the test could be read. The average stay is only two to three days, despite the hospital policy of a five-day stay for post-partum hospitalization.

Nineteen per cent of the 630 Blockley patients had never had chest X-rays taken; only one of the 308 patients at Woman's College Hospital had never had an X-ray taken. At Woman's College Hospital two asymptomatic nonreactors to tuberculin had significant previously unsuspected nontuberculous pulmonary disease. One had sarcoidosis, the other a congenital heart lesion. No previously unsuspected active tuberculosis was found.

Experience at both the small teaching hospital and the large city hospital revealed that the theoretical advantages of tuberculin testing may be outweighed by the administrative difficulties. Before relinquishing the gains available through routine chest X-rays of pregnant patients, due consideration should be given to the probability that, if routine procedures are used, the return rate for reading tuberculin tests may be as low as 50 per cent and that significant previously unsuspected

nontuberculous disease may be missed if only reactors receive chest X-rays.

Environmental versus Constitutional Factors in the Development of Tuberculosis among Negroes

JULIUS KATZ and SOLOMON KUNOFSKY, Albany, N. Y.

Identical environments are provided for white and Negro patients in the hospitals of the New York State Department of Mental Hygiene. Patients in these hospitals are fed, housed, and cared for without regard to race, so that differences in environment are practically nonexistent, and differences in tuberculosis morbidity and mortality may be ascribed to constitutional factors. Under such conditions, a close similarity in tuberculosis morbidity and mortality may be interpreted as evidence of the importance of nonconstitutional factors.

The results of the study may be summarized as follows:

1. Between 1942 and 1954 the incidence of tuberculosis among Negro patients in mental hospitals exceeded the rate among whites by about 50 per cent at most. In New York City during the same period, using case rates as approximations of incidence rates, the rate of development of tuberculosis was about four times as high among Negroes as among whites. During these years, the incidence rate in mental hospitals decreased at least as rapidly among Negro patients as among white, so that by the end of this period the age-adjusted rate among Negroes was only 8 per cent higher than among whites.

2. Even before the development of antimicrobial therapy, not only was the differential between the Negro and white rates reduced by similar environmental conditions, but the Negroes' survival was about equal to the whites'.

3. Antimicrobial therapy is as effective among Negro patients as it is among white patients.

In conclusion, under similar conditions of environment, the response to tuberculosis of the Negro is similar to that of the white, and racial factors probably play a minor role in producing differences in tuberculosis morbidity and mortality in the two races.

Tuberculosis Control in Northern Labrador

W. A. PADDON, North West River, Labrador, Canada

The probable origins of tuberculosis in Labrador Eskimos and Indians suggest that there is no special susceptibility or particular difficulty in treating these natives. The role of epidemics in propagating tuberculosis is described.

Death rates fell from 300 to 20 per 100,000 in 12 years and case incidence in surveys fell from 25 to 4 per cent.

Methods that won unqualified cooperation from a reluctant population are described. Portable X-ray equipment on a hospital ship was used, and follow-up work was done by winter dog teams until air transport became available. Hospitalization, drugs, surgery, and programs of rehabilitation and education were major tools of control. BCG was tried and found wanting.

Modern aircraft, radio communication, an excellent nursing staff and hospital facilities, and mission schools for health education as well as general education enabled the Grenfell Mission to pursue this program with government support.

Legal Custody of Recalcitrant Tuberculous Patients: Report on a Hospital Program

ROBERT H. BROWNING and IRVING PINE, Columbus, Ohio

The use of legal processes to hospitalize recalcitrant tuberculous patients is increasing in the United States. Because only a few reports have appeared in the medical literature, it appears worth while to discuss the philosophy, techniques, and results of 18 months of experience in the operation of a security division in a state tuberculosis hospital.

Comments will be made regarding some legal aspects of recalcitrant patient care, with comparison of the two major methods used to hospitalize these patients, namely, health department order and probate court commitment.

It is proposed to outline staffing policies and patient regimen and regulations in the context of results obtained. The origin of the patients—whether from rural or city homes—will be presented. Psychiatric findings will be classified and a study made of the personality types involved in this group of tuberculous recalcitrants. Results of therapy and the use of surgery will be correlated with type and extent of disease present.

3A Nontuberculous Pulmonary Disease

The Treatment of Eight Cases of Deep Mycotic Infection with Amphotericin B with Particular Emphasis on Drug Toxicity

HALLARD W. BEARD, JOEL H. RICHERT, and RICHARD R. TAYLOR, Denver, Colo.

Six cases of chronic pulmonary coccidioidomycosis, one case of disseminated blastomycosis, and one case of chronic pulmonary histoplasmosis have been treated intravenously with amphotericin B at Fitzsimons Army Hospital. Definite clinical improvement was attained in five of the cases of chronic pulmonary coccidioidomycosis and in the case of disseminated blastomycosis.

A detailed analysis of the toxic effects of amphotericin B was made. Albuminuria and cylindruria were frequently noted. A significant elevation of blood urea nitrogen occurred during each course of therapy. There was a corresponding inability to concentrate urine, excrete phenolsulfonphthalein dye, and clear blood urea and creatinine. All urinary abnormalities cleared following therapy.

Measurements of serum concentrations of amphotericin B indicate that therapeutic levels are maintained between periods of infusion. Amphotericin B was not detected in the serum after oral or intramuscular administration. At present, intravenous administration is the favored route.

Aspergillosis

SYDNEY M. FINEGOLD, DRAKE WILL, and JOHN F. MURRAY, Los Angeles, Calif.

Clinical, laboratory, and pathologic data are available from 12 cases of aspergillosis observed in our institutions during the past five years. Autopsies were performed on ten patients; the other two patients are still living. The presence in our series of several varieties of this disease and a survey of the literature have enabled us to derive the following classification.

I. Primary aspergillosis—in the absence of underlying disease or other predisposing factors.

A. Localized (abscesses or granulomata—including mycetoma; allergic—bronchitis with asthma and/or eosinophilia)

B. Invasive (acute—usually bronchopneumonic; chronic granulomatous disease—usually pulmonary)

C. Disseminated—widespread abscesses or granulomata

II. Secondary aspergillosis—associated with a local process, debilitating illness, impaired host defenses, or complicating antibacterial and/or steroid hormone therapy.

A. Localized—usually pulmonary (saprophytic relationship—abscess or mycetoma, focal infiltration; chronic, low-grade infection or granuloma)

B. Invasive—usually bronchopneumonic

C. Disseminated (endocarditis; multiple abscesses or granulomata in the absence of endocarditis)

There was one case of primary aspergillosis; the others represented the three categories of the secondary variety. All patients with secondary aspergillosis had debilitating underlying disease and had received antimicrobial therapy, usually multiple drugs for a prolonged period. Six of these patients had received adrenocortical steroids or corticotropin, and three were severely leukopenic. Three patients had associated candida infections.

Prophylaxis through judicious use of antimicrobial and steroid therapy is the most effective means of combating the increasing incidence of aspergillosis. Although usually ineffective, medical therapy has a greater chance of success in invasive and progressive forms of the disease when diagnosed early. Localized abscesses and granulomata are amenable to surgical resection.

In conclusion, a classification of aspergillosis is presented. Most cases are of the secondary variety associated with debilitating illnesses and prolonged antimicrobial or steroid therapy.

Clinical and Physiological Results following Radioactive Iodine (I^{131}) in the Treatment of Chronic Pulmonary Insufficiency

LOIS T. ELLISON, ROBERT G. ELLISON and WILLIAM F. HAMILTON, Augusta, Ga.

Twenty-three patients with severe pulmonary insufficiency, all badly disabled in spite of intensive and long-term therapy with conventional measures, were followed for two and a half to three and a half years after treatment with radioactive iodine (I^{131}). Complete clinical evaluation and pulmonary function studies were carried out before treatment and at two-month intervals thereafter.

The average total quantity of I^{131} per patient was 40.1 mc. with a range of 17.5 to 75.0 mc. An average of 1.9 doses was administered, with a range of 1 to 4 doses. Although hypometabolism was never a real problem and was usually easily controlled with 15 mg. or less of desiccated thyroid daily, 11 patients required thyroid therapy. One patient developed mild thyroiditis two weeks following treatment.

Clinical improvement was judged as excellent in seven patients (30 per cent), good in two (9 per cent), fair in six (26 per cent), and poor in eight (35 per cent).

Results of pulmonary function studies before and after treatment were prepared. No significant improvement in lung volumes or in the mechanics of breathing occurred. However, in the majority of patients, oxygen uptake and I^{131} uptake were reduced, total and alveolar ventilation decreased, alveolar and arterial oxygen tension increased, and carbon dioxide tension decreased. Comparison of certain significant physiologic responses with clinical improvement reveals that, although the same laboratory results were obtained in most of the patients, these did not always correlate with clinical improvement.

It is concluded that radioactive iodine may bring about symptomatic relief to the patient with severe pulmonary insufficiency who is refractory to the usual forms of therapy.

The Use of Intermittent Positive Pressure Breathing to Prevent Carbon Dioxide Retention Induced by Oxygen in Pulmonary Emphysema

RICHARD T. CATHCART and WILLIAM FRAIMOW, Philadelphia, Pa.

The administration of 100 per cent oxygen to patients with severe pulmonary emphysema often results in retention of carbon dioxide because of the attendant decrease in ventilation, and may cause a severe respiratory acidosis, narcosis, or even death. It is nonetheless frequently necessary to treat these subjects with oxygen in order to combat the severe hypoxia that may be present. This study was designed to determine whether the administration of 100 per cent oxygen by intermittent positive pressure breathing (IPPB), with its mechanical hyperventilation, could successfully prevent further carbon dioxide retention and correct the hypercapnea already present.

A group of 35 anthracosilicotics was studied. These patients had moderate to markedly severe obstructive emphysema as evidenced by maximum breathing capacity, vital capacity, and residual volume. Ventilation was measured and arterial blood sampled while the patient breathed ambient air, 100 per cent oxygen, and finally 100 per cent oxygen administered by IPPB. The arterial blood was analyzed for oxygen saturation, carbon dioxide content, and pH. The arterial partial pressure of carbon dioxide (pCO_2) and alveolar ventilation were calculated.

One hundred per cent oxygen breathing resulted in some degree of carbon dioxide retention in 32 of the 35 patients studied. The degree of retention was related to the severity of the emphysema. The significant increases in pCO_2 were associated with a decrease in ventilation. When 100 per cent oxygen was administered by IPPB, there was a reduction of the pCO_2 below control values in 32 cases. In every patient the mechanical hyperventilation successfully corrected the hypercapnea induced by the 100 per cent oxygen alone.

The results indicate that 100 per cent oxygen can be used safely in the treatment of severe pulmonary emphysema provided that adequate hyperventilation with IPPB can be established. In addition, the degree of carbon dioxide retention and the response to IPPB have been correlated with the severity of the emphysema. This tabulation permits an effective prediction of the individual patient's response to therapy.

The Effect of Helium and Oxygen Mixtures on Pulmonary Resistance in Emphysema

JOHN M. TYLER, ELIHU CHANNIN, and BIRGER GRAPÉ, Boston, Mass.

The clinical hypothesis that helium and oxygen mixtures are of benefit in obstructive disease of the lungs has been held for a number of years. Investigation of their efficacy, however, has been limited. Using standard methods for determining compliance of the lungs and resistance to air flow, we have evaluated the changes produced by the mixture of 80 per cent helium and 20 per cent oxygen in severe emphysema. Neither the dynamic compliance nor the functional residual capacity was altered. Inspiratory, expiratory, and total pulmonary resistances were reduced by about 30 per cent of the resting values when the subjects breathed helium at normal respiratory rates. At rapid respiratory rates, decreases of the same magnitude were produced except that there was no change in expiratory resistance.

It is believed that this decline in resistance is significant in the short-term treatment of severe emphysema.

Disturbances of Pulmonary Function in Fifty Patients with Bronchiectasis

NEIL S. CHERNIACK, KENNETH L. VOSTI, GEORGE A. SAXTON, JR., MARK H. LEPPER, and HARRY F. DOWLING, Chicago, Ill.

Thirty-two men and 18 women with evidence of bronchiectasis on bronchography were studied. They were from 16 to 75 years of age, with a mean of 48 years. In 82 per cent of the patients, the bronchiectasis was bilateral. In 40 per cent, emphysema was demonstrated by clinical or X-ray examination. Sixty per cent had had a cough for 10 or more years. Fifty-four per cent had a daily sputum volume exceeding 100 ml. Satisfactory tests of ventilatory factors were completed in all 50 patients, but diffusion and venous admixture data were adequate in only 35 of the patients studied. At least one test was abnormal in every patient.

The most striking finding was that of increased dead space. In 89 per cent of the cases the physiologic dead space exceeded normal, with a mean percentage of dead space to tidal volume of 49 per cent. This finding seems only logical, in view of the dilatations of the bronchi found in the bronchograms. The second most common abnormality was that of increased venous blood reaching the arterial circulation, occurring in 82 per cent of the cases. The mean venous admixture was 17 per cent, or approximately three times normal. This probably resulted from continuing blood flow through poorly ventilated alveoli. In 63 per cent of the patients the vital capacity and in 78 per cent the maximum breathing capacity were less than 80 per cent of normal. Mean vital capacity was 70 per cent of normal and mean maximum breathing capacity was 55 per cent of normal.

These data indicate that in almost all cases increased resistance to air flow reduced dynamic ventilation more than restriction of the lung

expansion or the loss of pulmonary parenchyma did. Furthermore, the reduction of maximum breathing capacity correlated better with the degree of dyspnea experienced by the patients than any other parameter measured. The distribution of inspired gas was nonuniform, as judged by nitrogen washout in 54 per cent of the patients. This disturbance was usually mild. The diffusing capacity of the lungs was below normal (9 ml. oxygen per minute per mm. Hg. per M.) in 34 per cent of the cases and did not correlate with increased venous admixture or dead space.

Goiter and Myxedema due to Iodide Therapy

BENJAMIN BURROWS, ALBERT H. NIDEN, and WILLIAM R. BARCLAY, Chicago, Ill.

Despite several reports of thyroid enlargement and hypothyroidism resulting from the therapeutic use of inorganic iodine, this syndrome is not widely recognized. Four such cases have been observed by the authors during the past year. One patient received iodides for chronic bronchial asthma, the others for chronic obstructive emphysema. All developed frank clinical myxedema. In three, thyroid function returned promptly to normal after discontinuing iodide therapy. The fourth was treated successfully with thyroid extract. In one subject with severe emphysema, withdrawal of potassium iodide resulted in a rapid disappearance of myxedema and a concomitant deterioration in cardiopulmonary status, leading to his death.

The described syndrome is apparently more common than generally thought. It must be recognized to avoid an erroneous diagnosis of intrinsic thyroid disease and to prevent the possible disastrous effects of sudden withdrawal of iodide therapy in severe pulmonary insufficiency states.

3B Tuberculosis Research

Aerial Dissemination of Pulmonary Tuberculosis: A Two-year Study of Contagion in a Tuberculosis Ward

RICHARD L. RILEY, C. C. MILLS, WALENTY NYKA, N. WEINSTOCK, PATRICK B. STOREY, L. V. SULTAN, MARY C. RILEY, and W. F. WELLS, Baltimore, Md.*

During this cooperative study between the Veterans Administration, The Johns Hopkins University School of Hygiene and Public Health, and the Maryland Tuberculosis Association, patients with far advanced pulmonary tuberculosis occupied the six single rooms of an experimental ward provided with a calibrated closed-circuit ventilating system and with a large animal exposure chamber located in the exhaust duct of this system. Approximately 156 guinea pigs were exposed continuously. Between November, 1956 and November, 1958, 71 animals contracted tuberculosis.

Evidence that these infections were air-borne from the patients on the ward includes the following: (*1*) the experimental design was such that the guinea pigs were not to our knowledge exposed to tuberculosis except by the aerial route; (*2*) since the characteristic lesion in the lungs of infected animals was a single tubercle, it seems unlikely that the pulmonary infection occurred by hematogenous or lymphogenous spread from a source elsewhere in the body; (*3*) strong evidence against cross-infection between guinea pigs is supplied by the random distribution of infections in the 36 different cages; and (*4*) in 21 instances the matching of the cultural characteristics of tubercle bacilli isolated from a guinea pig with those in the sputum of a patient occupying the ward at the time the guinea pig was infected provides presumptive identification of the specific patient who infected a specific guinea pig.

Over the two-year period the guinea pigs breathed, and hence sampled, over a million cubic feet of ward air. Since 71 quanta of air-borne tuberculosis were extracted by the guinea pigs from this huge volume of air, each quantum, on the average, was suspended in 15,000 to 20,000 cubic feet of ward air. All six patients together contributed on the average only 15 to 20 quanta of infection per day to the air of the system, according to calculations based on knowledge of dilution factors in the air of the system. There was tremendous variability in the aerial infectivity of different patients as indicated by the number of guinea pigs they infected. Awareness of the quantitative relationships involved in aerial dissemination of pulmonary tuberculosis should be helpful in devising control measures.

Sites to Which Intranasally Instilled Tubercle Bacilli Are Conveyed

HUGH E. BURKE, EDITH MANKIEWICZ, and JAMES R. STUART, Montreal, Canada

Previous studies have shown that intrapleurally introduced particulate matter is conveyed, in part at least, to the parasternal and the para-aortic lymph nodes.

Tubercle bacilli in suspension were instilled into the nares of 24 guinea pigs, 12 of which had been vaccinated with BCG. Animals were sacrificed at intervals of one to eight days. The pleural washings, the parasternal and para-aortic lymph nodes,

* This was a cooperative study by the Veterans Administration, The Johns Hopkins School of Hygiene and Public Health, and the Maryland Tuberculosis Association.

a retrotracheal node, and samples of liver and spleen were examined for tubercle bacilli by means of injection into other guinea pigs. Samples of lung were cultured on Löwenstein medium.

Three experiments have been carried out. These have shown that within 24 hours intranasally instilled tubercle bacilli are carried to (*1*) the lungs and/or the retrotracheal nodes, (*2*) the pleural spaces and/or the parasternal and para-aortic nodes, and (*3*) the liver and/or the spleen.

Tuberculin Allergy in Mice—Comparisons of Mouse Strains and Testing Techniques

ALFRED J. CROWLE, Denver, Colo.

Several techniques for detecting delayed hypersensitivity in mice have been reported. However, used in various laboratories on different mouse strains, they have not given consistent or agreeing results. The experiments reported in this paper were meant to evaluate some of these techniques under comparable conditions and to help answer various questions that have arisen concerning the alleged peculiarities of mouse tuberculin allergy.

Mice of seven different readily available inbred strains were sensitized by two weekly subcutaneous injections of H37Ra tubercle bacilli in w/o emulsion and tested for delayed hypersensitivity three weeks after the first vaccination by using six different methods.

Results indicated that essentially there was no difference in sensitization frequency among C57BL, C57BR, C57L, DBA, Swiss Webster, or CF#1 mice. However, C3H mice reacted less often. Among reactors of six strains there also was little variation in reaction intensity, but Swiss Webster mice responded more strongly as estimated by the objective intramuscular test.

Footpad, intracutaneous, and intramuscular tests were judged the best tests for detecting delayed hypersensitivity. Lung density, body temperature, and systemic tests proved more suitable as supplementary rather than primary methods. However, the lung density and body temperature tests have certain properties that should make them particularly useful in certain kinds of experiments.

The results of successful intracutaneous tuberculin tests were detailed, and evidence was presented that they are truly indicators of delayed hypersensitivity.

The Relationship of Serum Levels of a Lysozyme-like Enzyme to Sensitization of Guinea Pigs with M. Tuberculosis

BERNARD W. JANICKI and ROBERT A. PATNODE, Washington, D. C.

A marked elevation in the concentration of a lysozyme-like enzyme in rabbit serum following immunization with heat-killed tubercle bacilli has been reported (Myrvik and Weiser, 1951). Also, plasma levels of a similar substance have been found to be significantly increased in tuberculous patients (Kerby and Chaudhuri, 1953).

The results of our studies indicate that a similar situation prevails when guinea pigs are sensitized with heat-killed tubercle bacilli (H37Rv) suspended in paraffin oil. In a preliminary experiment, levels of a lysozyme-like enzyme were determined, by a modification of a method previously described (Kerby, 1952), in sera of 12 normal guinea pigs and 12 guinea pigs injected four months previously with heat-killed tubercle bacilli (5 mg.) in paraffin oil. Concentrations of enzyme were found to be significantly elevated ($P < 0.01$) in the sera of sensitized animals. In a subsequent study, 36 guinea pigs were injected with heat-killed tubercle bacilli (5 mg.) in paraffin oil. Sera were obtained from groups of four sensitized animals just prior to injection and at various intervals thereafter. A significant increase ($P < 0.01$) in serum enzyme activity appeared 10 days after sensitization and persisted throughout the study. Animals injected with paraffin oil alone exhibited no alteration in enzyme activity during a similar experimental period.

It is interesting to note that the increased serum levels of a lysozyme-like enzyme occurred at the time when skin sensitivity first became manifest and the "plasma factor" responsible for *in vitro* tuberculin cytolysis was demonstrable (Janicki, 1959). This observation and the fact that the leukocyte is generally considered to be a primary source of this enzyme (Tullis, 1953) make it tempting to believe that this increased activity is a reflection of some type of *in vivo* activity of tuberculoprotein on tuberculin-sensitive leukocytes. It has been suggested that a form of *in vivo* leukocytolysis may occur during the development of a skin reaction to tuberculin (Favour, 1951; Cole and Favour, 1955). However, no increase in enzyme levels was observed in sera from sensitized animals collected one, two, or three days following intradermal injection of 5 γ of PPD. The mean diameter of the skin reactions (48 hours) in the sensitized guinea pigs was 13.7 mm. of induration and erythema.

Additional studies are now in progress to elucidate further the relationship of serum levels of a lysozyme-like enzyme to the hypersensitive state.

The Effect of Fresh Human Serum on the Reaction of Mycobacteriophage D-29 with Certain Mycobacteria

ARTHUR WHITE, Louisville, Ky., and VERNON KNIGHT, Nashville, Tenn.

Fresh human serum at concentrations of 10 per cent and greater has been found to prevent propa-

gation of mycobacteriophage D-29 on both virulent and avirulent tubercle bacilli. Serum concentrations as high as 80 per cent did not prevent propagation of phage D-29 when the saprophytic Mycobacterium, ATCC 607, was used as the propagating strain. Sera from either tuberculin-negative or tuberculin-positive subjects or from patients with active tuberculosis prevented propagation on tubercle bacilli at the same concentrations.

The effect of serum was greatly diminished by incubation with trypsin but was not altered by heating at 56°C. for one hour, by incubation with tubercle bacilli or bacteriophage, or by dialysis. The active principle was precipitated by one-third saturation of serum with ammonium sulfate (the gamma globulin fraction).

The failure of serum to neutralize phage D-29 and to prevent propagation of ATCC 607 indicates that the site of action is on the tubercle bacillus and not on the mycobacteriophage. Since it was found that adsorption of phage on tubercle bacilli was not prevented by serum, the tubercle bacillus was apparently altered in such a manner that propagation was prevented at a stage after adsorption had occurred.

Cytology of Phage-Host Interactions in M. Tuberculosis

SMITH SHADOMY, SEYMOUR FROMAN, and M. JOHN PRICKETT, Los Angeles, Calif.

Changes in the intracellular structure and organization in *M. tuberculosis*, strain H37Rv, following infection with mycobacteriophage D-29, were studied by means of nuclear staining techniques and phase-contrast microscopy.

A progressive sequence of events involving definite changes in the chromatin structure was observed and associated with lytic phage infection; the events were assumed to be concerned with the replication of new phage DNA and the formation of mature phage particles. There was first a dispersion of the normal chromatin material and a definite reorganization into several large chromatin masses. These masses were observed to increase in size and intensity of staining during the remainder of the latent period until they were superseded by lysed or ghost cells.

The gross morphologic changes in the host cell following phage infection and lysis were recorded by phase-contrast microscopy.

The Induction of the Lysogenic State in M. Tuberculosis Strain H37Rv

RUTH L. RUSSELL, GREGORY J. JANN, Los Angeles, Calif., and SEYMOUR FROMAN, Olive View, Calif.

Attempts to find a naturally occurring lysogenic tubercle bacillus have thus far proved futile. However, the lysogenic state was induced in *M. tuberculosis* strain H37Rv by exposing it to a filtrate of mycobacteriophage D-32. The phage-host mixture was plated on Bordet-Gengou agar. Colonies surviving lysis were repeatedly subcultured and exposed to antiphage serum. A number of such colonies were found to produce phage and were also resistant to the phage produced.

A comparison of the cultural and biochemical characteristics and guinea pig virulence of the lysogenic strain with the parent strain revealed them to be indistinguishable.

An Enzymatic Basis for the Potentiation of Isoniazid by Para-aminosalicylic Acid

DAVID A. LEVY, WILLIAM S. SPICER, NICHOLAS R. BACHUR, and SAMUEL P. BESSMAN, Baltimore, Md.

Previous studies by Hughes have shown that the major metabolic product of isoniazid in man is the acetyl derivative, 1-isonicotinyl-2-acetylhydrazine. Johnson found that isoniazid is acetylated in the presence of pigeon liver extract and that para-aminosalicylate inhibits this acetylation. The inhibition was thought to be of the competing substrate type. Concurrent administration of PAS with isoniazid to humans by several groups of investigators resulted in elevation of serum concentration of the non-acetylated isoniazid.

Bessman and Lipmann have shown that pigeon liver extract catalyzed a low-energy acetyl transfer between various aromatic amines. In addition to the aromatic amines, hydrazine was shown to participate in this reaction. It was concluded that an acetylenzyme compound is formed as an intermediate step in the transfer:

$$\text{acetyl-arylamine} + \text{enzyme} \rightleftarrows \text{acetyl-enzyme} + \text{arylamine}$$

We have been able to demonstrate an acetyl transfer between isoniazid and PAS in the presence of pigeon liver extract:

$$\text{acetyl-isoniazid} + \text{PAS} \xrightleftharpoons{\text{enzyme}} \text{acetyl-PAS} + \text{isoniazid}$$

The formation of the various intermediates has been confirmed by several techniques, and quantitative observations will be reported.

It is suggested that PAS causes elevation of serum isoniazid level and decreases in level of acetyl-isoniazid by accepting acetyl from acetylated isoniazid.

The Influence of Isoniazid and Isoniazone Calcium Pyruvate on the Vitamin B_6 Metabolism of Rats

JENG M. HSU, PATRICK B. STOREY, and BACON F. CHOW, Baltimore, Md.

Japanese workers have reported their laboratory and clinical experience with a new analogue of isoniazid, Vivo-Niplen (VN), or isoniazone calcium pyruvate. Ito *et al.*, at Osaka University, have shown that the administration of VN to humans results in considerably less excretion of pyridoxine than equivalent doses of isoniazid do. It is their opinion that higher effective therapeutic doses of isoniazid can be accomplished by the use of VN with less neurotoxicity, and this without the necessity for concomitant administration of pyridoxine.

In this study, vitamin B_6-deficient male rats, with average body weight of 150 gm., were obtained by feeding a casein-sucrose diet supplemented with all known nutrients except B_6. The rats were divided into the following groups: (*1*) controls on the B_6-deficient diet alone, (*2*) those on the B_6-deficient diet plus supplemental B_6, (*3*) those on the diet plus isoniazid, (*4*) those on the diet plus VN, and (*5*) those on the diet plus supplemental B_6 and isoniazid.

The effects of B_6 deficiency alone, of B_6 deficiency plus isoniazid, and of B_6 deficiency plus VN on the general appearance of the animals, average weight gain following pair feeding, onset of convulsions, and exhibition of acrodynia were noted.

The urinary excretion of B_6 at the end of each seven days for three weeks was determined according to the microbiologic method of Stokes *et al.* (*J. Biol. Chem.*, 150: 17, 1943). The X-ray mutant of the fungus neurospora sitophila (299) was used as the test organism.

At the end of the three-week period all rats were sacrificed, and determination of B_6 content was done on the tissues, including blood, liver, kidney, and brain.

The results so far obtained indicate that there is a striking difference between the isoniazid-treated and the VN-treated animals, those receiving isoniazid showing a far greater urinary excretion of B_6 than those receiving VN. The results of the organ assays for B_6 are not yet available. It is hoped that these findings will throw some light on the role of isoniazid in producing or aggravating a vitamin B_6-deficiency state and at the same time yield useful information about the influence of Vivo-Niplen.

4A Respiratory Disease Research

Subjective and Objective Methods in the Diagnosis of Chronic Bronchitis

CHARLES M. FLETCHER, London, England

A clear conception of what is to be diagnosed is a necessary preliminary to diagnosis. In the case of chronic bronchitis this is no more than chronic bronchial inflammation of nonspecific etiology. This is a pathologic concept but, since it is difficult to obtain material for pathologic examination in life, one is forced to base diagnosis on the function consequences of the disease. The primary consequence is bronchial hypersecretion. Obstruction to bronchial airflow, causing dyspnea and a liability to recurrent bronchial infections, may be regarded as secondary to the primary hypersecretion and is not always present.

Bronchial hypersecretion is manifest clinically as production of sputum. This may be detected by symptomatic enquiry or by collection of sputum specimens. A comparison of these two techniques was made on 144 postmen. Questions were asked on two occasions concerning the time of day and year at which phlegm was produced, and specimens of sputum consisting of all that was expectorated in the first hour after rising were collected on two mornings in February and March; 86 per cent of the men returned the sputum bottles that were issued. Measurements of $FEV_{1.0}$ (forced expiratory volume) were also made. The answers to questions and the results of sputum collection were related, but the relation was imperfect. Those who denied having phlegm had the least sputum, those admitting to phlegm for only part of the day had significantly more, and those admitting to producing it throughout the day had significantly more again. A similar differentiation could be made between these three groups in respect of mean $FEV_{1.0}$ and frequency of chest illnesses. Those who admitted to perennial phlegm produced slightly, but not significantly, more sputum and had more illnesses than those admitting to winter phlegm only, but there was no difference in their mean $FEV_{1.0}$. There was complete correspondence between a simple triple classification of bronchitis according to answers to questions and volume of winter sputum in 63 per cent of the cases. The ability to repeat the two methods (over 70 per cent) was found to be similar.

It is suggested that bronchitis may be diagnosed and graded either by asking three questions on production of phlegm or by measurements of sputum volume. Both techniques are liable to observer and subject error and should, if possible, be combined. Other causes of productive cough are

so much less common that they need not be excluded in epidemiologic surveys.

The Effect of Ultraviolet Radiation upon the Transmission of Epidemic Influenza in Long-term Hospital Patients

Ross L. McLean, Atlanta, Ga.

The advent of Asian-strain influenza created new opportunities to study the mechanism of transmission of epidemic influenza. Prior work on this subject includes studies of ultraviolet radiation of barracks, recreation halls, and other places of congregation. These studies were interpreted as showing a "partial effect on certain types of respiratory disease" but not sufficient to justify a recommendation for general use. This study was based upon the concept of "aerial isolation" as described by William Firth Wells, which states that the principal mode of transmission of air-borne disease may be blocked by adequate disinfection of air that has been contaminated by the aerosolized respiratory secretions of infected persons. Ultraviolet radiation of the upper air of rooms has been shown to be effective in disinfecting truly air-borne particles, but relatively ineffective against surface contamination at lower levels. Thus, if disinfection of upper air of rooms diminishes the incidence of influenza, this effect must be related to the relative importance of air-borne mechanisms in the transmission of influenza.

An entire hospital building unit, housing approximately 150 patients, was totally radiated, and facilities for the total care of these patients, with no need to venture outside the radiated area, were provided. Samples of serum were obtained from all hospital personnel and all patients at the end of July, 1957, again in mid-November, 1957, and finally, in mid-March, 1958. Virtually none of the patients or personnel were vaccinated. During the first phase (July 28, 1957, to November 15, 1957), approximately 186 susceptible (by serology) patients resided within the radiated area. Only 2 (1 per cent) were infected by the transradiation route. By contrast, 11 (4 per cent) of 248 susceptible nonradiated patients and 56 (12 per cent) of 465 susceptible personnel showed serologic evidence of infection. During the second phase of the study (November 16, 1957, to March 15, 1958), of 277 susceptible patients within the radiated area, only 1 (0.4 per cent) case of transradiation infection occurred. By contrast, 41 (20 per cent) of 201 susceptible nonradiated patients and 35 (9 per cent) of 370 susceptible personnel showed serologic evidence of infection.

The striking differences in the incidence of infection, as determined by serologic means, between the radiated patients and the hospital personnel, in particular, suggests strongly that an important mechanism of transmission of epidemic influenza was significantly blocked. It is possible that an air-borne mechanism has been blocked by the aerial isolation accomplished by ultraviolet disinfection of the upper air of an entire hospital building unit.

Psittacosis and Q Fever in Laboratory Personnel

Allan H. Levy, Edward W. Hook, and Robert R. Wagner, Baltimore, Md.

Despite thorough safety precautions, laboratory personnel working with the causative agents of psittacosis and Q fever frequently become infected. Between 1945 and 1957, 12 cases of psittacosis and 27 cases of Q fever occurred sporadically in the microbiologic laboratories at Fort Detrick, Maryland. Eleven of the 12 patients with psittacosis had worked directly with the virus, but only two of these had been aware of a laboratory accident. The illnesses were similar to, but somewhat milder than, naturally acquired infections. Whereas eight patients had an abrupt onset with malaise, headache, chills, and fever, four had an insidious onset. Cough was common but often did not develop until the second week of illness. Rales were heard early in the course of illness in three patients but did not develop until the fourth or fifth day of hospitalization in six others. X-ray findings of pulmonary infiltration were noted in the lower or middle lobes of ten patients and in the upper lobes of two patients. Nine of the patients were treated with one of the tetracyclines, chloramphenicol, or penicillin, and all responded promptly.

In contrast to the psittacosis cases, only 18 of the 27 patients with Q fever worked directly with *C. burnetii*, although several others were infected while in areas adjacent to laboratories. In four, the source of infection remained obscure. None of the illnesses was severe, and only 21 of the 27 required hospitalization.

Although most patients had been vaccinated with both Q fever and psittacosis vaccines, complement-fixing antibody titers after infection were higher and persisted longer than those resulting from immunization. It is not possible to determine if the large proportion of mild illnesses was the result of prior immunization or mode of infection or the result of effective case detection through continual surveillance of a closed population.

The Effect of Oxygen Therapy on the Pulmonary Capillaries

BEN V. BRANSCOMB, BEULAH M. HATHAWAY, and CHARLES B. MCCALL, Birmingham, Ala.

Recently evidence has been presented supporting the hypothesis that inhalation of oxygen in therapeutic concentrations induces pulmonary capillary proliferation, sometimes after only two days. Because of the importance of this hypothesis in relation to the clinical use of oxygen, two experiments were conducted. First, seventy-six mice were divided into three groups. One group breathed room air, the second was exposed to compressed air in a sealed chamber, and the third breathed 57 ± 2 per cent oxygen. Animals were sacrificed from each group every two days. After fourteen days, all remaining animals were returned to room air, and sacrifices continued for fourteen additional days. Each lung served as a control for the opposite lung. The sections were coded and interpreted "blind." No evidence for an oxygen effect could be found.

Seventy-four consecutive human autopsies have been studied to date. The identity of the subject and the oxygen history were withheld from the pathologists until the sections had been studied. Since patients currently in the hospital were used, careful records of the method and duration of oxygen therapy could be obtained. Correlation of the histologic change with the mode and duration of oxygen therapy yielded the following conclusions:

1. Some degree of focal capillary proliferation occurred in 78 per cent of the subjects.

2. Thirty-two subjects showed moderate or severe capillary proliferation, but of these patients 19 received no oxygen therapy.

3. There was no correlation between the duration of oxygen therapy or the method of its administration and the presence or severity of capillary proliferation.

4. The occurrence of focal capillary proliferation did not seem to depend on the presence of cardiopulmonary disease at the time of death but was seen in many subjects with otherwise unremarkable lungs.

4B Experimental Methods

Lung Structure in Three Dimensions after Inflation and Fume Fixation

B. J. BLUMENTHAL and HOLLIS G. BOREN, Houston, Texas

The unique lability of the lung prevents its being cut without gross distortion. As the elaborate techniques devised to prevent this distortion have not come into common usage, a simple method of fume fixation is presented.

The lung is inflated and fixed by air containing formaldehyde fumes. The inflated specimen is rigid, dry, and nonfragile. New methods of cutting, staining, and mounting allow the entire architecture from gross appearance to the details of cellular structure to be seen. Three-demensional structure is visualized by stereoscopic microscopy.

The results are: (*1*) human emphysema may be studied to classify the types and distribution of lesions and to identify early changes; (*2*) morphologic changes may be correlated with physiologic alterations present before death; (*3*) mechanical properties of the lung may be studied prior to fixation; (*4*) the form and arrangement of alveoli are demonstrated; (*5*) the pattern of pulmonary capillary bed is well defined; (*6*) the method provides an excellent tool for screening lungs of experimental animals so that the results of air pollution are visualized.

Preparation of Normal and Emphysematous Lung Tissue for Structural Studies of Bronchioles, Alveolar Ducts, and Alveoli

PHILIP C. PRATT, Columbus, Ohio

A major problem in the study of the anatomy and pathology of the smaller respiratory structures is their marked tendency to collapse and become distorted during fixation and preparation for examination. This is especially true in the presence of any type of emphysema in which a variable degree of distortion is present *in vivo*, but there is difficulty in identifying it in the presence of artefactual distortion. Furthermore, finer structures are so tortuous in three dimensions that it is rare to see complete longitudinal sections in ordinary preparations.

A method has been devised for the preparation of pulmonary tissue dried in a state of inflation comparable to conditions within the chest. Briefly this involves placing the lung in a specially designed respirator box and causing it to "breathe" formaldehyde vapor for about 18 hours in order to fix it. This is followed by about one week of steady inflation, which results in drying of the entire lung. When dry, the lung will maintain its size and contour indefinitely. Multiple sections with a thickness of about 1 mm. have been cut from apex to base of a series of lungs thus prepared. The large area of cut surface makes possible the frequent visualization of longitudinal sections including all structures from bronchioles to alveoli. Sections have been made from normal and emphysematous lungs.

Another of the problems relating to the identification of structural changes in obstructive emphy-

sema is the rarity with which lungs become available for study from patients known through previous pulmonary function studies to have obstructive emphysema. For this reason a further extension of the present study involves a demonstration of the ventilatory activities of the excised lung. It is believed that rather simple studies of expiratory flow rate and patterns will suffice to identify examples of obstructive emphysema in lungs that may be obtained from such patients who have not had previous pulmonary function studies. It is a relatively simple matter to conduct such ventilation experiments using the lung respirator that is required for the inflation and fixation of the lungs.

Studies of the Elastic Tissue of the Lung

ROBERT W. CARTON, BEVERLY TEWS, JOHN DIANAUSKAS, and GEORGE M. HASS, Chicago, Ill.

Elastic tissue occurs as a major connective tissue component of the human lung, and much of the retractile force of the lung has been ascribed to its action. In the past a very few serial reconstructions of pulmonary elastic tissue have been assembled so that this network could be studied in three dimensions, but because of the difficulty of this method most descriptions have been based upon conventional thin microscopic preparations. We have taken advantage of the selective resistance of pulmonary elastic tissue to concentrated formic acid and have isolated this network by dissoiving other elements of lung structure from around it. In this manner the elastic tissue of many lungs can be compared with ease, and the structural background of pulmonary behavior can be examined.

Blocks of lung measuring about 1.5 cm. in all dimensions are taken during autopsy and placed in 88 per cent formic acid for 72 hours. They are then neutralized and stained with basic fuchsin. Only a thin delicate network remains, which outlines the pattern of the former pulmonary structure. This elastic network can be suspended in water for microdissection or can now be embedded in gelatin or plastic.

Blocks from fifty normal lungs have been treated by this method, and slides and photomicrographs of the important elastic structures are available. The following points are striking in these preparations: (*1*) the richness of the elastic network of the distal air spaces; (*2*) the continuity of elastic fibers between the pulmonary vasculature, pulmonary parenchyma, and pleura; and (*3*) the manner in which the air passages can be traced by the elastic network from bronchiole to alveolus.

Three lungs from persons suffering from severe hypertrophic pulmonary emphysema were examined, and the sparse and irregular elastic tissue structure and the increased thickness and staining irregularities of many of the remaining elastic fibers were noted.

The Erythropoietic Factor in Hypoxic Patients with Emphysema without Secondary Polycythemia

JAMES F. HAMMARSTEN, WALTER H. WHITCOMB, ROBERT M. BIRD, PHILIP C. JOHNSON, and MARGARET MOORE, Oklahoma City, Okla.

Many patients with chronic hypoxia due to pulmonary emphysema fail to develop a secondary polycythemia.

This study is concerned with the biologic assay for erythropoietin activity in the plasma from such patients. The experimental plasmas were donated by five patients with obstructive emphysema. Each patient had hemoglobin values distinctly below the level that would be expected in a normal individual chronically exposed to a similar degree of hypoxia. Plasmas donated by two young men in vigorous, good health served as controls. All plasmas were heated to 98°C. for 10 minutes, and the supernatant fluid was dialyzed and reconstituted to the original plasma volume. Mature male Sprague-Dawley rats were free-fed and injected intraperitoneally daily for 13 consecutive days. The daily dose of plasma filtrate injected was equal to 2 per cent of the animal's current weight. The blood values were obtained immediately prior to the first injection and the day following final injection. Each group of rats receiving hypoxic plasma filtrates showed a mean increase in hemoglobin values and erythrocyte counts. Rats receiving normal plasma or saline showed a variable response. The increases in hemoglobin and erythrocyte counts exhibited by the animals given hypoxic plasma were significantly different from the control value at the $P = <0.02$ level. A similar trend was noted in the hematocrits.

These results indicate that the plasma of patients who are hypoxic as a result of pulmonary emphysema but who have not developed secondary polycythemia contains a quantity of erythropoietin greater than that seen in normal subjects. This is interpreted as additional evidence that their erythropoietic unresponsiveness results from a refractory bone marrow.

A Constant Volume Fractional Sample Collector for Repetitive Cardiac Output Determinations

MARGARET E. GANTT, M. MOSTAFA ABDEL-FATTAH, and DAVID W. CUGELL, Chicago, Ill.

Continuous recording methods have greatly simplified cardiac output determinations but re-

quire much larger doses of radioactive substances or of special dyes than multiple sample techniques. These large dose requirements limit the number of times the cardiac output can be measured with safety. If multiple samples are collected, the handling and manipulation of each sample is burdensome, particularly when several flow determinations are necessary.

A new type of fractional sample collector has been designed to aid in cardiac output determinations. This device will collect as many as 60 consecutive, discrete arterial blood samples of constant volume and at any rate up to 0.5 seconds per sample. The samples are transferred directly to automatic scintillation counting equipment. The sample size may be as large as 0.7 ml. and the volume per sample is constant regardless of variations in arterial blood pressure. Radioactive iodinated albumin is used as the indicator.

The apparatus has been tested in the following three ways:

1. Continuous flow through a mechanical model. Flows were determined simultaneously by direct measurement and were calculated from the fractional sample collector. In 18 trials with a flow range of 1,190 to 3,010 ml./min. the radio-iodine calculated flow was from 93 to 104 per cent of the measured flow. The mean difference was 3 per cent.

2. Measurements with a dual pump system that provided recirculation. A timed-concentration plot of injected tracer was indistinguishable from curves obtained from patients. In 13 trials, with flows from 1,845 to 4,120 ml./min., the radio-iodine measured flow percentage range was 80 to 105 per cent, with an average difference of 7 per cent.

3. Simultaneous direct Fick and fractional sample radio-iodine output determinations. In 25 comparisons in dogs the range of Fick cardiac output was 520 to 2,260 ml./min. The radio-iodine/Fick percentage was 81 to 142 per cent, with an average difference of 10 per cent.

In all three methods agreement was good. As little as 2 to 8 mc. of radio-iodine was sufficient for cardiac output determinations in man, and it can be repeated with little or no increase of indicator dose.

A Rapid Mouse Test for the Diagnosis of Pulmonary Tuberculosis

David Gale, Elizabeth A. Lockhart, and Alexander Jack, Albuquerque, N. M.

Preliminary studies with known positive patients indicate that inoculation of mice with patients' specimens combined with an adjuvant is much more rapid in yielding evidence of tubercle bacilli in such specimens than is found by the usual cultural procedures. An evaluation of 1,000 routine specimens by both mouse inoculation and culture is now being done. The results obtained on the first 470 specimens indicate that the mouse test is not only more rapid than culture procedures but also more sensitive. Many patients considered negative by culture have been found to be positive by mouse test and are excreting viable tubercle bacilli.

4C Tuberculin Testing

PPD Intermediate: Its Diagnostic Significance in Active Tuberculosis

Sol Katz, Washington, D.C., Robert C. Duvall, Baltimore, Md., Thomas J. Ryan, Thomas F. O'Connor, R. J. Marilley, and Richard B. Perry, Washington, D. C.

The intermediate PPD skin test has been considered the most valuable screening skin test in the diagnosis of tuberculosis. Reports have recently stated that this test will be negative in a significant number of active tuberculosis cases, which limits seriously its diagnostic value. This paper presents a critical reappraisal of its specificity in active tuberculosis in a two-part prospective type of study. The necessity of repeating the test in initial negative or doubtful reactors before considering the patient truly tuberculin negative is emphasized.

Commercially available PPD intermediate antigen was used in 300 cases of bacteriologically and/or histologically proved active tuberculosis. The test was routinely repeated immediately in all initial negative or doubtful reactors. Over-all results show the test to be positive in 294 of 300 cases. Five of the six remaining patients had obvious cause for anergy. Excluding these, the test was positive in 99.7 per cent. Of the 300 patients, 7 per cent were initially negative but were positive upon immediate retesting.

The Variability of Tuberculin Tests

Emil Bogen and George Altbach, Olive View, Calif.

Differences in tuberculin tests responsible for reports of tuberculin conversions or reversions are often misleading, due to variations in the performance or reading of the tests or to nonsignificant fluctuations in tissue sensitivity. The frequency and significance of such chance variations were studied by performing multiple tests, simultaneously and successively, on the same series of subjects and obtaining multiple independent readings of the results by different observers. Reading error in more than 1,300 paired readings averaged only about one millimeter, but the variation between the diameter of the reactions to the same

dose of tuberculin placed in both forearms at the same time by the same or different workers was more than twice as great and resulted in an excessive number of apparent discrepancies interpreted as conversions or reversions. The causes for some of the discrepancies were investigated, and measures for preventing some of them developed. It was concluded, however, that for reliable results in a study of tuberculin tests, each test should be performed at least twice before a negative report is accepted.

The Tuberculin Test and the Diagnosis of Clinical Tuberculosis

DAVID T. SMITH, WILLIAM W. JOHNSTON, HERBERT A. SALTZMAN, and J. HARNED BUFKIN, Durham, N. C.

The reactions to second-strength PPD are true allergic responses and not the result of chemical irritation from tuberculin. Healthy individuals reacting exclusively to second-strength PPD have a resistance to the development of clinical disease appreciably greater than those giving 20 mm. or more reaction to five TU and definitely greater than those who fail to react to second-strength PPD.

Some of the second-strength reactions are caused by infection with atypical acid-fast bacilli or other organisms and some are caused by tubercle bacilli. The exact proportions of each remain a matter of controversy.

The size of the dose of tuberculin needed in diagnostic studies and the size of the reactions depend not only on the inherent degree of allergy but also on the physiologic capacity of the skin to react. Patients with chronic progressive pulmonary tuberculosis, as well as those with miliary tuberculosis or meningitis, may have such a reduction in tissue tone that they react only to second-strength PPD, or they may fail to react even to this large dose.

In a well-fed, stabilized sanatorium population, 99.6 per cent of the patients with proved tuberculosis may give a positive reaction to the five-TU dose. However, sicker patients are not so reactive. In one state sanatorium, where the patients were tested on admission, 50 (8.7 per cent) of 624 who eventually proved to have tuberculosis failed to react to five TU, and 4 (0.6 per cent) did not react to second-strength PPD. In the Durham VA Hospital 5 (6.1 per cent) of 81 proved cases failed to react to five TU, and one did not react to second-strength PPD. Duke Hospital does not deliberately admit medical cases of tuberculosis. Among 120 proved cases 20 (16.7 per cent) failed to react to 1:1000 OT but were positive to 1:100 OT, and 18 (15 per cent) did not react to 1:100 OT.

The percentage of tuberculous patients who react to five TU varies with the type of hospital and with the degree of illness of the patient.

Experiences with the Newer Tuberculin-testing Techniques: Patch, Modified Patch, Disk, Heaf, etc.

MICHAEL L. FURCOLOW, LAWRENCE E. WOOD,* and IRENE L. DOTO, Kansas City, Kans.

It appears an appropriate time to summarize experiences with the newer tuberculin-testing techniques compared with the intradermal tuberculin test employing intermediate-strength PPD. These comparisons have been done over the past five years and include hundreds of persons, both children and adults.

An attempt will be made to summarize the advantages and disadvantages of the various testing techniques.

Comparison between Purified Individual Tuberculoproteins and the International Standard Tuberculin, PPD-S

H. M. VANDIVIERE, Chapel Hill, N.C., FLORENCE B. SEIBERT, Philadelphia, Pa., and MARGARET R. VANDIVIERE, Chapel Hill, N. C.

Several purified tuberculoproteins—A, B, C, and a bacillary fraction—have been compared as skin-test antigens in individuals vaccinated with several BCG strains (1,000) or with an attenuated human strain, R_1Rv (600), in naturally converted non-tuberculous groups (1,200), and in tuberculous patients (1,400). The reaction to the equivalent dosage in micrograms of PPD-S served as a base line. Concentrations tested were 0.00002, 0.0001, and 0.005 mg. Statistical analyses have been made comparing each group as to quantitative volume of reaction elicited and the numbers of reactors detected.

Test antigens were injected intradermally into each forearm, each person acting as his own control. The volume of the reactions was measured and calculated as the segment of a sphere. Complete statistical analysis has been made for each group, individually and comparatively.

In general, comparative testing with PPD-S and Fraction A results in slight differences. "A" is, however, greatest in the number and percentage of reactors after vaccination with R_1Rv. Fraction B proves to have the greatest sensitivity and specificity. Fraction C is significantly weaker than the others. The bacillary is greatest in volume in vaccinated and naturally converted nondisease groups but is significantly less than PPD-S, "A," or "B" in tuberculous patients.

* Deceased.

It appears (*1*) that the skin reactions to these several fractions may well indicate immunity with one pattern of response (A ≅ or > PPD:B > PPD:C not sig.: Bac. > PPD) and disease with another (A ≅ or < PPD:B > PPD:C not sig.: Bac. < PPD); (*2*) also, of clinical interest, that a "B" fraction demonstrates a sensitivity and specificity that is far superior to either PPD or OT (and may answer some of the nonspecificity problems).

The results of precipitin tests with these various fractions in rabbit sera (vaccinated and tuberculous) are suggestive of correlation with animal prognosis, electrophoretic pattern, and is not contradictory to the tuberculin response data in humans.

Corticotropin-induced Changes in the Tuberculin Skin Test: A Controlled Study in Advanced Tuberculosis

HARVEY SALOMON and JOSEPH H. ANGEL, Brooklyn, N. Y.

A controlled study of the effects of corticotropin on the early course of moderately and far advanced pulmonary tuberculosis has recently been concluded. Cases that met the criteria for admission to the study were randomly assigned to either the corticotropin or the control group. The former group, in addition to triple antituberculosis chemotherapy (isoniazid 0.3 gm., PAS 12 gm., and streptomycin 1 gm. daily), received corticotropin gel beginning at 80 units and decreasing to a maintenance dose of 30 to 40 units daily for three months. The preliminary report on the first 80 patients to complete this trial has been presented elsewhere. The clinical course, X-ray clearing, sputum conversion, pulmonary function, and other aspects of the two groups were compared.

As a simultaneous but independent smaller study, 63 of the randomized patients had their tuberculin reactions studied. Five tuberculin units of PPD-S (0.0001 mg. of international standard PPD) were given intradermally to each patient before treatment and during the sixth week, the third month, and the sixth month of treatment. Differences in the reaction to tuberculin between the group given corticotropin and the control group were then statistically analyzed.

There is a significant reduction in the extent of the tuberculin reaction under the influence of corticotropin. At six weeks, all control patients had positive reactions by American Trudeau Society standards. At the same time, among those patients receiving corticotropin, eight had no induration at all and ten had negative reactions.

Since corticosteroids may be expected to have a comparable effect, caution is recommended in the interpretation of tuberculin tests in patients receiving the steroids or corticotropin. The reduction in sensitivity to tuberculin further suggests the use of corticotropin or the steroids in those cases of tuberculosis in which toxicity is a major consideration, e.g., acute hematogenous tuberculosis, tuberculous meningitis, and far advanced pulmonary tuberculosis.

5A Immunology

The Role of BCG Vaccination in the Elimination of Tuberculosis

ARMAND FRAPPIER, Montreal, Canada

(Abstract not available at time of publication)

BCG in Chicago: A Twenty-year Study Statistically Analyzed

SOL ROY ROSENTHAL, ERHARD LOEWINSOHN, MARY L. GRAHAM, DOROTHY LIVERIGHT, MARGARET G. THORNE, VIOLET JOHNSON, and H. C. BATSON, Chicago, Ill.

The study was initiated in 1937 with newborn infants who were from nontuberculous households, who were born at the Cook County Hospital in Chicago and who lived in high tuberculosis prevalence areas. Some 3,383 infants, vaccinated or not, born from 1937 to 1948, were followed to 1957.

The follow-up of the children included birth weight, sex, race, area of birth, examinations in the clinic, X-ray, tuberculin tests, extent of contact with tuberculosis, tuberculin conversion rates, etc. These and other factors totaling some eighty items were card-catalogued, tabulated, and submitted for statistical analysis.

The statistical analyst, Dr. Batson, states: "The over-all comparability of the vaccinated and the control subjects appears adequate in most regards. Statistically significant differences have been identified for consideration and assessment for medical importance.

"The difference in the number of cases of tuberculosis among the control subjects (57 in 1,665) and the vaccinated (16 in 1,716) was statistically highly significant ($\chi^2 = 24.62$ p = <0.001)."

The Evaluation of a Serum Gel-Diffusion Precipitation Test for the Diagnosis of Tuberculosis

ROBERT C. PARLETT and GUY P. YOUMANS, Chicago, Ill.

An extensive cooperative double-blind study of the usefulness of a gel-diffusion precipitation test for the diagnosis of tuberculosis has been in prog-

ress since July 1, 1958. This program has involved the Department of Microbiology, Northwestern University Medical School, and 17 cooperating hospitals and sanatoriums in various parts of the country. The major goals set for this program were: (*1*) to determine the sensitivity, reliability, and limitations of the gel-diffusion precipitation test for the detection of antibodies in the serum of tuberculous persons; (*2*) to determine the specificity of the test; and (*3*) to determine the relationship between the presence of circulating antibodies as detected by the gel-diffusion test and tuberculin hypersensitivity.

It is anticipated that complete data will be available by May, 1959, on sera obtained from between 2,500 and 3,000 patients.

Preliminary data (as of January 1, 1959) based on the results of tests performed on approximately 1,000 specimens of serum indicate that slightly more than 90 per cent of patients with bacteriologically proved tuberculous disease will show a positive gel-diffusion test. On the other hand, only about 5 per cent of persons with clinically diagnosed nontuberculous disease have shown a positive gel-diffusion test. Furthermore, over 90 per cent of tuberculin-positive but clinically nontuberculous individuals do not show a positive gel-diffusion test.

A complete and detailed analysis of the clinical and laboratory data on the larger number of patients and an evaluation of the sensitivity, reliability, and validity of the technique have been prepared. In addition, a study of the gel-diffusion test as used for the detection of antibodies in tuberculous and nontuberculous simian sera has been analyzed, and the effect of BCG vaccination on the production of antibodies detectable by the gel-diffusion technique has been studied.

A Correlation of the Results of the Agar Double-diffusion Precipitation Test with the Bacteriological Findings in 210 Consecutive Patients

WILLIAM LESTER, JR. and ROSEMARY COLTON, Hinsdale, Ill.

Sera from 210 consecutive patients admitted to a tuberculosis hospital were studied for the presence of antimycobacterial antibodies by means of the agar double-diffusion precipitation. The test was found to give reproducible and consistent results. In this series there were 129 patients with bacteriologically confirmed diagnoses of tuberculosis, and 123 of them (95.3 per cent) were found to be antibody positive. All (100 per cent) of the 25 cases of photochromogenic and five cases of Battey atypical infection were antibody positive. However, only 82 per cent of patients with only scotochromogenic atypical cultures were found to be antibody positive. There were 33 patients in the series who had demonstrable pulmonary pathology but who were culture negative throughout the period of their observation, and only 60 per cent of them had demonstrable antibody. A comparison of the rate of tuberculin reactors to five TU of PPD with the presence or absence of antimycobacterial antibodies strongly suggested that the results of agar double-diffusion precipitation tests were independent of tuberculin sensitivity.

A Correlation of the Results of the Agar Double-diffusion Precipitation Test with the Bacteriological Findings in 189 Consecutive Patients

WILLIAM LESTER, JR. and ROSEMARY COLTON, Hinsdale, Ill.

(Abstract of paper not available)

5C Thoracic Surgery

Surgical Experience and Histopathologic Findings in Pulmonary Infections Caused by Photochromogenic Mycobacteria

GEOFFREY KENT, ROBERT W. HARRISON, WILLIAM LESTER, ARTHUR F. REIMANN, EDWIN T. LONG, and WILLIAM E. ADAMS, Hinsdale, Ill.

Analysis of the results of resectional surgery in 22 patients with pulmonary disease caused by photochromogenic mycobacteria revealed an undue frequency of postoperative complications in these cases. The resected specimens of 10 of these cases were carefully studied, and the findings compared with those seen in similar specimens, matched as to extent and character of disease, obtained from patients infected with typical human type tubercle bacilli.

The tissues from the photochromogenic cases demonstrated the whole gamut of anatomic and histopathologic findings seen in typical tuberculosis. However, they also exhibited certain differences. Severe involvement of the bronchi was a prominent feature in every case, and it appeared to be a process that grew by extension and contiguity with few distant foci. Furthermore, the process was characterized by a dearth or, in some instances, a complete lack of fibrous encapsulation. Schaumann bodies and asteroids were frequently seen, and fat phagocytosis was a prominent feature.

The relation of these histopathologic findings to the increased risk, observed after wedge or segmental resections in patients with photochromogenic mycobacterial infections, was investigated.

Operative Occlusion of the Bronchus in the Treatment of Selected Cases of Pulmonary Tuberculosis

J. Maxwell Chamberlain, New York, N. Y., Peter Parnassa, Brooklyn, N. Y., and Thomas M. McNeill, New York, N. Y.

It is an established pathologic fact that one method of healing in cavitary tuberculosis is the result of inflammatory occlusion of the broncho-cavitary junction. The endoscopic application of caustics, both experimentally and clinically, has failed to mimic this natural occlusion. Surgical division of the bronchus serving the diseased area, however, will successfully imitate this pathologic process.

Tuberculous cavities cultured at the time of Monaldi drainage were universally free of secondary organisms. Consequently, operative occlusion of the associated bronchus is well tolerated. Broad-spectrum antimicrobials used preoperatively provide additional security.

The most frequent common denominator among the resection failures was the inability to obliterate residual dead space, e.g., after resection of the left upper lobe together with the superior segment of the left lower lobe. Operative occlusion of the bronchus, on the other hand, avoids the usual complications of resectional therapy. Furthermore, the pulmonary function is preserved, and the sputum is converted immediately.

Our experience is based upon a small series of cases during the last 10 years. One death occurred and was not related to the operation. No complications have occurred. The cases are described in the paper and, although the indications for the operation are few, it is conceivable that the usefulness of the procedure will increase when the results of long-term antimicrobial therapy have been established. We believe the procedure is indicated especially for the emphysematous patient with negative sputum and persistent "ground shadows" in the chest roentgenogram.

Intrathoracic Mesothelioma

Donald B. Effler and Carl A. Broaddus, Jr., Cleveland, Ohio

Tumors arising from the mesothelial lining of the thorax are not common. During the past ten years, 13 proved cases have been studied at the Cleveland Clinic; one of this series may be of peritoneal origin. In each case tissue studies were obtained, some form of therapy recommended, and complete follow-up studies established.

In the series of 13 patients, four tumors were classified as benign, nine as malignant. One of the four benign cases manifested epithelial elements as well as fibrous; on the basis of published reports this constitutes a histopathologic rarity.

Pulmonary osteoarthropathy is considered by many to be almost pathognomonic of mesothelioma. According to most published reports, it is at least mentioned as a common accompaniment of mesothelioma. Therefore, it is of particular interest that in none of the 13 cases here reported was pulmonary osteoarthropathy present.

Subjective and objective findings in patients with mesothelioma are not specific. The significant clinical features and the methods of diagnosis in this series of cases are described.

The four benign cases were treated by surgical excision, and there have been no recurrences. The nine malignant intrathoracic mesotheliomata were treated with surgery, nitrogen mustard, radiation, or combinations of the three. All patients but one were dead within two years of onset of symptoms. The sole survivor, treated by pneumonectomy and decortication, appears to be in good health two-and-a-half years after onset of symptoms.

Coarctation of the Aorta as Associated with Other Cardiovascular Anomalies

William J. Kuzman and Albert B. Klug, San Diego, Calif.

This report concerns 20 consecutive patients who were referred with a tentative diagnosis of coarctation of the aorta. On subsequent examination, the diagnosis was confirmed in 18 patients. The other two cases were diagnosed as pulseless disease.

Of the 18 cases of coarctation of the aorta, 16 were of the adult type and two were of the infantile type. In 12 cases surgical repair of the coarctation was carried out with excellent results. Four asymptomatic patients under age three with an adult type of coarctation are continuing under observation. One patient with associated ventricular septal defect and patent ductus arteriosus died nine days after birth, and one with a ventricular septal defect is now being prepared for surgery. The group (16) with the adult type of coarctation had significant associated cardiovascular defects in ten instances, as did both the patients with the infantile form.

There were nine males in this series and nine females. The youngest patient with coarctation of the aorta was nine days old and the oldest 43 years old. Four of the surgical repairs were carried out under hypothermia because of associated cardiac defects.

Electrocardiographically, all patients with essentially pure coarctation of the aorta demonstrated a normal tracing or only minimal suggestion of left ventricular hypertrophy. All patients

with associated cardiac abnormalities demonstrated grossly abnormal electrocardiograms.

Orthocardiograms demonstrated rib-notching in six of 14 patients. The one patient with an infantile type of coarctation demonstrated the classical unilateral rib-notching. Roentgenographically, patients with uncomplicated coarctation had normal cardiac silhouettes, in contrast to those with associated cardiac defects.

Cardiac catheterization, angiocardiography, and/or pressures at surgery were carried out in seven of the eighteen patients.

The purpose of this report is to stress the high incidence of congenital cardiac anomalies associated with coarctation of the aorta. Similarly, the presence of a grossly abnormal electrocardiogram and/or orthocardiogram in a patient with suspected coarctation of the aorta should be a stimulus for an exhaustive study in order to rule out possible associated cardiac defects.

Evaluation of Clinical Condition in the Seriously Ill and Critical Effect of Small Overdosage in Medication

RALPH ADAMS and ROBERT CORELL, Wolfeboro, N. H.

This report summarizes the observations and presents the conclusions from careful, intensive study of a group of cases under conditions of nearly total monitoring in the premedication, preoperative, surgical, and early postoperative periods, and directs attention to the need for several forms of care likely to be disregarded. The data make clear the two facts that an astute physician can do most of these things from intuition but that measuring devices that record the changes at onset can be very helpful, especially as most details of care must be rendered by secondary personnel.

Total reactivity was followed in the cases of ten patients from the time before they received preoperative medication, and all of their homeostatic mechanisms were recorded before going to the surgical pavilion, throughout the operative period, thence to the recovery room, and eventually back to their own beds. Some of the findings were surprising. Small details about amount of medication are of decisive importance in the critically ill and in those facing surgery of large magnitude. Overmedication, especially of the debilitated, is commonly although unwittingly practiced. Need for special nursing attention was shown to be almost as critical in the preoperative time as it is in the postoperative period. The poor condition of some patients during early phases of operation was shown to be caused by changes not recognized by previous methods of study.

Hypoventilation is the most frequent of all abnormal causes for variations in cardiovascular function, especially in reference to the serious sequellae that it causes. The effects of deficient exhalation of carbon dioxide are marked on stroke volume output of the heart but delayed in time. Acidosis of serious extent can develop within a few minutes of a small dose of pentobarbital, and the deleterious effects of this variation can be manifested over 30 minutes of time, even though the hypoventilation caused by giving of the pentobarbital was corrected within two minutes of occurrence. Minor displacements of intestinal viscera or pressure upon organs, as by retractors, have been shown to cause immediate changes in the characteristics and caliber of the pulse wave by as long as three minutes before changes in clinical condition appear. Excessive ventilation is less damaging to homeostasis than is deficient ventilation, but it too is harmful. Deficient oxygenation from any one of several causes can exist for many minutes before it is recognizable clinically in the form of cyanosis, although its existence can be recognized immediately by oximeter readings and secondarily by effects upon stroke volume, cardiac rate, end-alveolar output, and pulse pressure before even astute and experienced observers can see cyanosis.

A system of recording has been evolved whereby all information can be put onto one sheet of paper simultaneously and with a great reduction in the amount of mechanical gadgetry necessary. The data recorded in these cases studied included blood pressure readings, end-alveolar carbon dioxide, constant arterial oxygen saturation, arterial and venous blood pH, stroke volume output, cardiac rate, respiratory tidal volume, minute ventilation, electrocardiogram, and temperature.

6A Symposium on Smoke, Smoking, and Chest Diseases

Statistical Associations Between Cigarette Smoking and Pulmonary Diseases

E. CUYLER HAMMOND, New York, N.Y.

(Abstract not available at time of publication)

A Critical Examination of Statistical Evidence Linking Cigarette Smoking with Chest Diseases

ABRAHAM M. LILIENFELD, Baltimore, Md.

(Abstract not available at time of publication)

The Localization of Inhaled Foreign Matter in the Respiratory Tract

A. C. HILDING, Duluth, Minn.

(Abstract not available at time of publication)

Pathogenesis of Pulmonary Disease Due to Chemical Environmental Agents

PAUL KOTIN, Los Angeles, Calif.

Epidemiologic studies have established that chemical and physical agents in our respiratory

environment are associated with clinical pulmonary diseases. One of these is lung cancer. Carcinogenic aromatic polycyclic hydrocarbons have been identified in urban polluted air and in the condensate of cigarette smoke. In addition, experimental carcinogenic agents belonging to the group of aliphatic hydrocarbon oxidation products have been demonstrated in the air. Extracts from these environmental sources have been used in the induction of skin tumors and mesenchymal sarcomas in a variety of mammalian species. Pulmonary tumors have also been induced in mice and rats following prolonged exposure to an atmosphere of ozonized gasoline.

The clinical distribution of pulmonary cancer is compatible with a combined role for polluted air and cigarette smoke in the pathogenesis of this disease. In addition to the carcinogenic agents, a large number of irritants have been demonstrated in polluted air and cigarette smoke. Though these chemical irritants are in themselves noncarcinogenic, they appear to have a role in the development of lung cancer. One of their properties is the ability to interfere with the normal flow of mucus on the respiratory epithelium. Under physiologic conditions, the continuous flow of the mucous stream tends to preclude the accumulation and prolonged retention of carcinogenic particulate matter on the respiratory epithelium. The slowing or cessation of mucous flow facilitates the localization and retention of these particulates. This serves the carcinogenic process in two possible ways. First, it permits abnormally long apposition of retained particles to the underlying epithelium and, second, it provides an extended time period for the elution of carcinogenic agents from soot particles to which they are adsorbed.

The response of the epithelium to irritants is apparently a nonspecific one in that a broad group of chemically unrelated compounds is capable of producing a slowing of the mucous stream. A significant factor in the production of this effect is the physical state of these chemical agents in that, when present as particulates, the effect is more intense and occurs at lower concentrations than that seen following exposure to gas phase material.

Over and above the presence of carcinogenic agents in polluted air and cigarette smoke, our experimental data demonstrate that these environmental agents by virtue of their interference with normal function of the respiratory epithelium qualify for a role in the pathogenesis of pulmonary cancer. Further, the ubiquity of these environmental agents and their etiologic compatibility with the epidemiologic data relating to lung cancer place the problem of their significance in the area of contribution to and responsibility for the observed increased incidence. Not to suggest a role for them is irreconcilable with currently available information.

An Experimental Approach to the Effects of Tobacco Smoke on the Lungs

CECILIE LEUCHTENBERGER, Boston, Mass.

(Abstract not available at time of publication)

Pathological Changes Observed in the Bronchi Associated with Smoking

OSCAR AUERBACH, East Orange, N. J.

(Abstract not available at time of publication)

A Critical Examination of Experimental Data on Smoking and Chest Diseases

ROBERT C. HOCKETT, New York, N. Y.

(Abstract not available at time of publication)

Certain Research Accomplishments and Research Needs in the Lung Cancers

DEAN F. DAVIES, New York, N. Y.

(Abstract not available at time of publication)

NOTICES

The following dates and locations of the next three annual meetings of the National Tuberculosis Association and the American Trudeau Society have been selected and approved:

1960—Los Angeles, California—May 15–20
1961—Cincinnati, Ohio—May 21–26
1962—Miami Beach, Florida—May 20–25

XV CONFERENCE OF INTERNATIONAL UNION AGAINST TUBERCULOSIS

The International Union Against Tuberculosis will hold its XV International Conference in Istanbul, Turkey, September 11–18, 1959. Preliminary programs are available at the Executive Offices of the National Tuberculosis Association.

Since its founding in 1920 and particularly in the past decade, this biennial conference has become an outstanding event and an authoritative source of information for all interested in any aspect of tuberculosis control. As is apparent in the list of scientific sessions below, the scope has broadened to include peripheral topics as well as strictly medical. Our Councillors have been in favor of this expansion as the Union is a federation of voluntary tuberculosis associations and can mitigate toward a better understanding of this concept in every country where an association exists. Several of the members, in addition to having accepted chairmanships will be speakers.

Details of travel, et cetera, can also be procured from the Executive Office of the National Tuberculosis Association, and anyone interested in attending is urged to write for both program and information.

Scientific Sessions

Tuesday, 15th September

9 a.m.–1 p.m.

SYMPOSIUM I: Chemoprophylaxis of tuberculosis—its experimental, clinical and social aspects.

Chairman: Professor Robert Debré (France).

3 p.m.–6 p.m.

SYMPOSIUM II: The problem of acid-fast bacilli (ecology, pathogenicity for man, bacteriologic characteristics).

Chairman: Professor Daniel E. Jenkins (U.S.A.).

Wednesday, 16th September

9 a.m.–1 p.m.

SYMPOSIUM III: Present day indications of surgical treatment of pulmonary tuberculosis.

Chairman: Sir Clement Price Thomas (Great Britain).

3 p.m.–6 p.m.

SYMPOSIUM IV: New antibacterial drugs.

Chairman: Professor L. Hailmeyer (Germany).

Thursday, 17th September

9 a.m.–1 p.m.

SYMPOSIUM V: The problem of relapse in all of its aspects (relapse after chemotherapy, surgery, collapse therapy, ambulatory treatment, and its importance from the epidemiologic standpoint).

a) Relapse after medical treatment:

Chairman: Professor M. Variéty (France).

b) Relapse after surgical treatment:

Chairman: Dr. R. S. Mitchell (U.S.A.).

Friday, 18th September

9 a.m.–1 p.m.

SYMPOSIUM VI: New methods in rehabilitation of the tuberculous.

Chairman: Dr. K. Oppikofer (Switzerland).

3 p.m.–6 p.m.

SYMPOSIUM VII: Health education in the light of new methods of treatment of tuberculosis.

Chairman: Dr. Tevfik Gokçe (Turkey).

SYMPOSIUM VIII: The role of the public health nurse and the social worker in tuberculosis control.

Chairman: Professor Jadwiga Szustrowa (Poland).

A Seminar on "Recent Knowledge on the Mechanism of Acquired Immunity in Tuberculosis," under the chairmanship of Professor R. Cruikshank (Great Britain), will be held on Friday, 18th September, 1959.

The role of BCG vaccination in the different countries of the world will be discussed on Saturday morning, 19th September, under the chairmanship of Professor Armand Frappier (Canada).

Preliminary planning indicates that the following NTA-ATS members will take part in scientific symposia:

Chemoprophylaxis of Tuberculosis

Walsh McDermott, M.D.
Carroll E. Palmer, M.D.
Leon H. Schmidt, Ph.D.
Mrs. Shirley Ferebee

The Problem of Acid-fast Atypical Bacilli

Daniel E. Jenkins, M.D. (Chairman)
Ernest H. Runyon, Ph.D.
William Steenken, Jr., M.D.

Carroll E. Palmer, M.D.
Seymour Froman, Ph.D.

Present-Day Indications of Surgical Treatment
Robert R. Shaw, M.D.
John W. Streider, M.D.
N. J. Wilson, M.D.

New Antibacterial Drugs
William B. Tucker, M.D.
William R. Barclay, M.D.
Gladys L. Hobby, Ph.D.
Walsh McDermott, M.D.
Leon H. Schmidt, M.D.
William Steenken, Jr., Ph.D.

The Problem of Relapse
(1) After Medical Treatment
H. McLeod Riggins, M.D.
William B. Tucker, M.D.

(2) Relapse after Surgical Treatment
Roger S. Mitchell, M.D.
James E. Raleigh, M.D.

Health Education in Light of New Methods of Treatment
Walsh McDermott, M.D.
James G. Stone
Albert M. Kessler

The Role of The Public Health Nurse and The Social Worker in Tuberculosis Control
Jean South, R.N.

New Methods of Rehabilitation of the Tuberculous
David T. Carr, M.D.

Recent Knowledge on the Mechanics of Acquired Immunity
Sidney Raffel, M.D.
Guy P. Youmans, M.D.

INDEX OF AUTHORS

Adiao, Amparo C., 31
Affronti, Lewis F., 284
Allen, Albert R., 680
Allison, Stanton T., 102
American Trudeau Society, 108, 118, 258, 263 387, 394, 397, 549, 684, 687, 690, 695, 697, 822, 851
Anghelis, B., 522
Aronson, David L., 83
Aronson, Joseph D., 731

Bachman, Henry, 87
Barclay, William R., 543, 678
Bartmann, K., 97
Bastarrachea, Fernando, 246
Baumgartner, Leona, 687
Beck, Frederick, 134
Becklake, Margaret R., 457
Bentley, Francis J., 756
Berte, Stephen J., 344
Berthrong, Morgan, 221
Blaker, Robert G., 152, 180
Blankenberg, Herman W., 357
Blazsik, C. F., 773
Blumenthal, B. J., 764
Bojalil, L. F., 246
Boren, H. G., 764
Breite, Melvin J., 672
Brouet, G., 6
Buehler, Edwin V., 622, 631
Bunn, Paul A., 72
Burke, Hugh E., 52
Burrows, Benjamin, 543

Canetti, Georges J., 684
Carabasi, Robert J., 543
Carneiro, Jose Fernando, 544
Carpenter, Charles M., 374
Chang, Y. T., 673, 805
Chevallier, J., 6
Choremis, C., 522
Cohen, Aaron A., 253
Collin, E., 484
Comstock, George W., 542
Conge, G., 484
Crisalli, Joseph P., 531
Cushing, Ivan E., 315
Custer, Edward W., 378

Derbes, Vincent J., 251, 531
Dewlett, Hal J., 344
Diena, B. B., 816
Dixson, Shirley, 492
Douthit, Vera B., 543
Doy, C. H., 492
Drake, Clifford L., 374
Dubos, Rene J., 80, 484
Dunbar, Frank P., 669
Dwork, Ralph E., 427

Edwards, Phyllis Q., 83
Eidus, L., 816
El Nagah, A. M., 119

Farid, Z., 119
Feraru, Felix, 577
Fergus, Emily B., 659
Frappier, Armand, 296
Freund, Julius, 87
Friedman, Bernard L., 265
Friedrich, T., 351
Frostad, Simon, 597

Gastambide-Odier, Mireille, 94
Gemmill, C. L., 339
Gerstl, B., 212
Gittens, S., Aubrey, 307
Glaser, Stanley, 427
Goldman, H. I., 457
González-Mendoza, Amado, 246
Green, Robert A., 790
Greenberg, L., 816
Greer, J. W., 119
Grumbach, Françoise, 1

Hall, Wendell H., 518
Hamilton, Mary Alice, 221
Hammarsten, James F., 606
Harris, H. Wm., 663
Hayashi, Mitsuo, 371
Herring, Jack L., 251, 531
Hiltz, J. E., 468
Holin, Sabine M., 427
Hollander, A. G., 212
Holm, Johannes, 690
Honska, Walter L., Jr., 606
Hudgins, Paul C., 323, 382
Huggin, Perry M., 204

Inada, Kiyoshi, 232
Ishak, K. G., 119

Jackson, Joan K., 659
Jacobs, Sydney, 105, 251, 531
James, H. A., 541
Janicki, Bernard W., 244
Jefferies, Mildred B., 669
Jeker, K., 351
de J. Macias, José, 265

Karns, James R., 746
Katsumura, Tatsuki, 232
Kaufman, Kiesl K., 525
Kawai, Keizo, 232
Khan, I., 474
Kitazawa, Yukio, 329
Konno, Kiyoshi, 810
Kraus, William, 731

Lauener, H., 351
Lawrence, Carl A., 374
LeGolvan, P. C., 119
Leifheit, Howard C., 344
LeMeur, G., 6
Levy, David, 666
Levy, F. M., 484
Levy, Richard S., 152, 180
Liacacos, D., 522
Libermann, David, 1
Limes, Barney J., 606
Lincoln, Edith M., 31
Linn, Richard H., 251, 531
Lorriman, Gerard, 756
Low, Eugene, 612
Lull, George F., Jr., 641
Lunn, Joseph, 72
Lupini, Belardino, 307
Lurie, Max B., 152, 180
Lyon, Richard H., 518

McAlister, Elizabeth, 669
Macias, José de J., 265
Mais, Edward L., 307
Marche, J., 6
Mark, Donald D., 440
Mihaly, John P., 307
Miller, William F., 315
Misener, F. J., 468
Mitchell, Mildred B., 533
Montalbine, Vincent, 66
Montes, Mario, 362
Morrow, Charles S., 512, 577
Morton, J. W., 474
Mousa, A. H., 119
Moyer, R. E., 90
Myers, J. Arthur, 19
Myrvik, Q. N., 339

Nagah, A. M. El, 119
Nakano, Akinori, 232
Naylor-Foote, A. W. C., 374
Newman, Robert W., 204
Noda, Yo, 371
Nutter, J. E., 339

Ohta, Shigeo, 329
Organick, Avrum B., 799

Orinius, Erik, 450
Ostrom, C. A., 541

Paley, Samuel S., 307
Patnode, Robert A., 323, 382
Pecora, David V., 41, 134, 679
Pérez-Tamayo, Ruy, 246
Petersdorf, Robert G., 238
Phillips, Charles, 362
Phillips, Samuel, 273
Pines, A., 818
Pongor, Ferenc, 652
Portelance, Vincent, 296
Puzik, V. I., 497

Quinlan, J. J., 468

Ramsay, J. H. Rolland, 818
Regli, J., 351
Renzetti, Attilio D., Jr., 72
Rheins, Melvin S., 622, 631
Rikli, Arthur E., 427
Rist, Noel, 1, 6
Rockey, E. E., 773
Rosenthal, Sol Roy, 105
Rubbo, Sidney D., 492
Runyon, Ernest H., 663
Russell, William F., Jr., 666

St.-Pierre, Jacques, 296
Sbarra, Anthony J., 810
Schwartz, William S., 90
Schwarz, Ch., 97
Seaman, James B., 681
Selin, Merle J., 663
Shelton, Neil W., 273
Shope, Robert E., 238
Smith, D. W., 94
Sochocky, S., 502
Spain, David M., 591
Sproule, Brian J., 315
Ståhle, Ingvar, 450
Staskiel, L. J., 512
Steenken, W., Jr., 66
Stepanyan, E. S., 142
Stocklen, Joseph B., 427
Strain, Anne K., 47
Stuart, Douglas G., 253
Suter, Emanuel, 47

Tani, Junkichi, 738
Taplin, George V., 374
Taylor, Richard R., 641
Terai, Takeo, 738
Thompson, S. A., 773
Thurston, J. R., 66

Townsend, Samuel M., 677
Tsukamura, Michio, 371
Tucker, Elon B., 344

Uvarova, O. A., 497

Verhoeff, Dirk, 357
Villnow, J., 97
Virach, Zoltan, 652

Walsh, John J., 251, 531
Wayne, Lawrence G., 526
Webb, Watts R., 780
Weiss, David W., 813
Weiss, William, 537
White, F. Clark, 134
Wiley, L. J., 541
Wolochow, H., 541
Wright, Kenneth W., 72
Wright, R. R., 212

Yamamoto, Masakuni, 371
Yamamura, Yoshirire, 738
Yamamura, Yuichi, 738
Young, R. C., 468
Yu, Paul N., 265

Zappasodi, Peter, 152, 180

INDEX OF SUBJECTS

Alaska, histoplasmin sensitivity in natives of (Notes), 542
Alcoholism in the tuberculous before and during hospitalization (editorials), 659
Alpha-ethyl-thioisonicotinamide
  antituberculous
    activity of, experiments on, 1
    effectiveness of, in tuberculosis in humans, 6
Altitude and respiratory function, 457
Alveolar cell carcinoma, 502
American Trudeau Society
  Annual Meeting, May 24–29, 1959: Abstracts of medical papers presented at, 822–850
  Manual for consecutive case conference (Pembine Type), 258
  Notices, 118, 263, 397, 549, 697, 851
  Obituaries, 118, 394, 695
  Organization and committee structure, 1958–1959, 108
  "Tuberculosis: A World-Wide Problem" conference, November 18, 1958, papers from, 684, 687, 690
Amylase content of pleural fluid in pancreatitis and other diseases, 606
Antigens, PPD and others prepared from atypical acid-fast bacilli and *Nocardia asteroides*, 284
Antimicrobials. *See* Chemotherapy
Artery(ies)
  innominate and subclavian, aneurysms of (Case reports), 790
  pulmonary, agenesis in circulation of (case reports), 641
Atelectasis, segmental, in children with tuberculosis, primary, 597

Bacillus(i)
  acid-fast, atypical, PPD and other antigens prepared from, 284
  tubercle. *See* Tubercle bacillus(i)
BCG
  method of obtaining (correspondence), 105
  -vaccinated
    guinea pigs, sonic fragility of leukocytes from, 323
    tuberculous children, serum protein electrophoretic pattern and Middlebrook-Dubos titer in (Notes), 522
  vaccination
    of mice, microscopy and culture of *M. tuberculosis* in, 484
    in Sweden (correspondence), 678
  vaccine
    new method of counting viable organisms in (Notes), 816
    standardization of, progress toward (editorial), 80
Blebs, subpleural, surgery of, 577
Books
  Annual Review of Medicine, Vol. 9. Edited by David A. Rytand, 256
  Antibiotics Annual 1957–1958. Edited by Henry Welch and Felix Marti-Ibanez, 256
  Cerebrospinal Fluid Production, Circulation, and Absorption. Ciba Foundation Symposium. Edited by G. E. W. Wolstenholme and Cecelia M. O'Connor, 385
  Chirurgie d'Exérèse dans la Tuberculose Pulmonaire. By D. Honoré, 546
  Chronic Bronchitis, Emphysema and Cor Pulmonale. By C. H. Stuart-Harris and T. Hanley, 546
  Clinical Enzymology. Edited by Gustav J. Martin, 106
  Die Entwicklung der Tuberkulosebehandlung seit 100 Jahren. By G. Domagk, 682
  Pasteur Fermentation Centennial 1857–1957. A Scientific Symposium on the Occasion of the One Hundredth Anniversary of the Publication of Louis Pasteur's *Mémoire sur la fermentation appelée lactique*, 384
  Practical Allergy. By M. Coleman Harris and Norman Shure, 384
  Rehabilitation After Illness and Accident. Edited by Thomas M. Ling and C. J. S. O'Malley, 820
  Respiratory Disease and the General Practitioner. By C. W. C. Toussaint, 106
  Selected Experiments in Medical Microbiology. By Stewart M. Brooks, 107
  La Souche du BCG. By A. Frappier and M. Panisset, 819
  Tracheotomy: A Clinical and Experimental Study. By Thomas G. Nelson, 256
  Tuberculosis in Animals and Man: A Study in Comparative Pathology. By John Francis, 682
  Über den Einfluss von Physikalischen und Chemischen Faktoren auf di Cytologi der Tuberkelbazillen und Anderer Mycobakterien. By Werner Roth, 548
Bronchograms under hypnosis (Notes), 525
Bronchus(i), regenerative versus atypical changes in, 591
Brucella suis, vaccines from gamma-irradiated, and from *M. tuberculosis* (Notes), 374

Calcium benzoyl-PAS and calcium PAS, tolerability of, 351

Carcinoma
alveolar cell, 502
of lung, primary, with tuberculosis, pulmonary, 134
Case finding
in psychiatric hospitals, resurvey interval of (Notes), 537
tuberculin test for in a general hospital, pilot study of (Notes), 378
Catalase activities of isoniazid-susceptible and -resistant strains of *M. tuberculosis* (Notes), 669
Chemotherapy
healing processes in, 497
in pneumoconiosis complicated by tuberculosis (correspondence), 818
and tuberculin sensitivity in rabbits, 329
in tuberculosis, 492
primary, in childhood, segmental lesions in, 756
Chromogens, acid-fast, in gastric juice of nontuberculous patients (correspondence), 543
Coccidioidin skin reaction in pulmonary coccidioidomycosis (case reports), 78
Coccidioidomycosis. *See* Mycoses
CORRESPONDENCE
BCG vaccination in Sweden, 678
Chemotherapy in pneumoconiosis complicated by tuberculosis, 818
Diffuse interstitial pulmonary fibrosis and hypertrophic pulmonary osteoarthropathy, 543
On the frequency of acid-fast chromogens in gastric juice of nontuberculous patients, 543
Method of obtaining BCG, 105
Plea for clearer distinction between allergic granulomatosis and Wegener's granulomatosis, 544
Rehabilitation and occupational therapy in tuberculosis hospitals, 680
Sophistry in use of the word "minimal," 681
Surgical reporting, 679
Treatment failures, 105
Vocational rehabilitation in tuberculosis, 543
Culture and microscopy of *M. tuberculosis* in BCG-vaccinated mice, 484
Cycloserine
and isoniazid in tuberculosis, pulmonary (Notes), 87
and viomycin in tuberculosis, pulmonary (Notes), 90
Cytolysis, leukocyte, "plasma factor" in (Notes), 244

Diabetes insipidus, pulmonary histiocytosis X with (case reports), 652
Douglas Bag in maximal breathing capacity, with spirometry (Notes), 253
Drug(s)
ancillary, in resection of drug-resistant cavitary tuberculosis, 780
therapy, preresection, in tuberculosis, pulmonary, 41

EDITORIALS
Progress toward the standardization of BCG vaccine, 80
Tuberculous alcoholic before and during hospitalization, 659
Effusions, pleural, tuberculous, prednisone in, 307
Enzymes
to aid filtration of oropharyngeal washes (Notes), 541
serum, in tuberculosis, pulmonary (Notes), 251
Erythrocytes, OT-sensitized sheep, and trypsinized human, serologic relation of, 622

Fenestration, tracheal, evolution and early results of, 773
Fibrosis, pulmonary, interstitial, and osteoarthropathy, hypertrophic (correspondence), 543
Fistula, bronchoesophageal, in tuberculosis, mediastinal (case reports), 238
Follow-up study of tuberculosis, with and without chemotherapy, 612
Fume fixation of lung, 764

Granulomatosis, allergic and Wegener's, distinction between (correspondence), 544

Healing processes in chemotherapy, 497
Helium-dilution method in ventilation study, 450
Histiocytosis X, pulmonary, with diabetes insipidus (case reports), 652
Histoplasmin
sensitivity
in Alaskan natives (Notes), 542
urban focus of (Notes), 83
Hormones, hydrocortisone acetate ointment, topical, at site of intracutaneous tuberculin reaction (Notes), 666
Hospital, tuberculous patients in, adjustment on different wards, 273
Hospitals, tuberculosis, rehabilitation and occupational therapy in (correspondence), 680
Hydrocortisone. *See* Hormones
Hyperplasia, lymph node, of mediastinum (case reports), 232
Hyperthyroidism in tuberculosis, native resistance to, 152
Hypnosis, bronchograms under (Notes), 525
Hypothyroidism in tuberculosis, native resistance to, 180

Immunology and pulmonary diseases, 212
Influenza, Asian, in 1957, pathology of, 440
Isoniazid
and cycloserine in pulmonary tuberculosis (Notes), 87
effect on growth of tubercle bacilli from pulmonary lesions (Notes), 518
hydrazones in biologic fluids, 492
in resistance, acquired (Notes), 97
serum-free, chemical and biologic determination method, 344
-susceptible and -resistant *M. tuberculosis* strains, catalase and peroxidase activities of (Notes), 669
toxic psychosis from (case reports), 799
in tuberculosis
experimental, reinfection in (Notes), 246
pulmonary, with pyrazinamide or PAS (Notes), 102

Kanamycin
in murine leprosy (Notes), 673
in tuberculosis, human, 72
in vitro and in guinea pigs, antituberculous activity of, 66

Leprosy
murine
evolution of (Notes), 805
kanamycin, streptovaricin, paromomycin, novobiocin, and ristocetin in (Notes), 673
Lesion(s)
pulmonary, isoniazid effect on growth of tubercle bacilli in (Notes), 518
segmental, in primary tuberculosis in childhood, 756
Leukocyte(s)
from BCG-vaccinated guinea pigs, sonic fragility of, 323
cytolysis, "plasma factor" in (Notes), 244
Lipids, extraction of, biologic properties of mycobacteria after, 296
Lung(s)
arterial circulation of, agenesis in (case reports), 641
carcinoma of, primary, with tuberculosis, 134
diseases of
chronic, and tuberculosis, respiratory function in (Soviet translation), 142
immunologic aspects of, 212
function
in resection, bilateral, 468
in stenosis, mitral, 265
in tuberculosis, pulmonary, 474
mucormycosis of (case reports), 357
schistosomiasis of, chronic, 119
structure in three dimensions after inflation and fume fixation, 764
Lymph node, giant, hyperplasia of mediastinum (case reports), 232
Lymphatics as drainage for pleura, parietal and visceral, 52

Maximal breathing capacity, spirometric and Douglas Bag measurement comparisons (Notes), 253
Mediastinum hyperplasia, lymph node, of (case reports), 232
Medical students, tuberculosis in, at University of Maryland, 746
Medium, egg, cage-laid, elimination of precleaning of (Notes), 677
Microscopy and culture of *M. tuberculosis* in BCG-vaccinated mice, 484
Middlebrook-Dubos titer and serum protein electrophoretic pattern in BCG-vaccinated tuberculous children (Notes), 522
"Minimal," sophistry in use of the word (correspondence), 681
Monocytes, immunity studies with, 221
Mucormycosis. *See* Mycoses
Mucosa, bronchial, regenerative versus atypical changes in, 591
Mycobacteria
biologic properties after lipid extraction, 296
growth rates of, in biochemical studies (Notes), 94
neutral red reactions of (Notes), 526
niacin test in distinguishing (Notes), 663
paratubercle bacilli, skin reaction to products of, 731
Mycobacterium
tuberculosis
H37Ra strain of, mechanical agitation in growth of (Notes), 813
isoniazid-susceptible and -resistant strains of, catalase and peroxidase activities of (Notes), 669
"plasma factor" in leukocyte cytolysis in guinea pigs sensitized with (Notes), 244
vaccines from gamma-irradiated, and from *Brucella suis* (Notes), 374
X, infectivity and immunogenicity of, in mice, 47
Mycoses
coccidioidomycosis, pulmonary, coccidioidin skin reaction in (case reports), 78
mucormycosis of lung (case reports), 357

Niacin test
in differentiation of tubercle bacilli (Notes), 810
in distinguishing mycobacteria (Notes), 663
Nocardia asteroides, PPD and other antigens prepared from, 284
Nodules, pulmonary, found in community roentgenographic survey, 427

NOTES
Acquired resistance and isoniazid medication, 97
The best doctor in the hospital, 533
Bronchograms under hypnosis, 525
Catalase and peroxidase activities of isoniazid-susceptible and -resistant strains of *M. tuberculosis*, 669
Circulating levels of the "plasma factor" responsible for *in vitro* leukocyte cytolysis during sensitization of guinea pigs with *Mycobacterium tuberculosis*, 244
Comparison of spirometric and Douglas Bag measurements of maximal breathing capacity, 253
Cycloserine and viomycin in the treatment of pulmonary tuberculosis, 90
Determination of growth rates of atypical strains and of other mycobacteria as a means of estimating optimal growth periods for comparative biochemical studies, 94
Differentiation of human tubercle bacilli from atypical acid-fast bacilli. III. Modification of the niacin test, using Tween-albumin liquid medium, 810
Distinguishing mycobacteria by the niacin test, 663
Effect of isoniazid upon growth of tubercle bacilli from pulmonary lesions, 518
Effects of kanamycin, streptovaricin, paromomycin, novobiocin, and ristocetin on murine leprosy, 673
Effect of mechanical agitation on the growth of the H37Ra strain of *M. tuberculosis*, 813
Effect of topical hydrocortisone acetate ointment at the site of intracutaneous tuberculin reaction, 666
Elimination of precleaning cage-laid hens' eggs in preparation of egg fluid for tuberculosis bacteriologic media, 677
Evolution of murine leprosy, 805
Experimental reinfection in arrested guinea pig tuberculosis and its behavior under isoniazid therapy, 246
Failure of polyoxyethelene ether (Triton WR 1339) to protect against tuberculin shock in guinea pigs, 382
Genetic considerations of the mechanisms involved in PAS-resistant tubercle bacilli, 371
Histoplasmin sensitivity in Alaskan natives, 542
Measurements of activity of ambulatory hospitalized tuberculous patients and the effects of a tranquilizing agent, 531
A new method of counting the viable organisms in BCG vaccine, 816
Pilot study of the tuberculin test for case finding in a general hospital, 378
Preliminary report on vaccines prepared from gamma-irradiated *Mycobacterium tuberculosis* and *Brucella suis*, 374
Pyrazinamide in low dosage in combination with isoniazid or para-aminosalicylic acid in the treatment of pulmonary tuberculosis, 102
Quantitative aspects of neutral red reactions of typical and "atypical" mycobacteria, 526
Report on the use of isoniazid and cycloserine in 84 cases of pulmonary tuberculosis, 87
Serum enzymes in pulmonary tuberculosis: Glutamic oxalacetic transaminase and glutamic pyruvic transaminase, 251
Serum protein electrophoretic pattern and Middlebrook-Dubos titer in tuberculous children following BCG vaccination, 522
Tuberculosis case finding in psychiatric hospitals, 537
Urban focus of histoplasmin sensitivity, 83
Urine test for the detection of PAS in ambulatory tuberculous patients, 672
Use of enzymes to aid filtration of oropharyngeal washes through membrane filter, 541
Novobiocin in murine leprosy (Notes), 673

OBITUARIES
Aronson, Joseph David, 695
Barrier, Leonidas F., 394
Brady, Edwin Herms, 394
Brumfiel, Daniel M., 396
Brzozowski, Grover S., 394
Cooley, Samuel S., 118
Gray, Frederick James, Jr., 118
Lange, Horst, 395
Schantz, John Philip, 394
Seldon, Frank G., 395
Sloan, E. F., 395
Walker, William Dunn, 394
Occupational therapy and rehabilitation in tuberculosis hospitals (correspondence), 680
Oropharyngeal region, enzymes to aid filtration of washes from (Notes), 541
Osteoarthropathy, hypertrophic, and fibrosis, pulmonary, interstitial (correspondence), 543
Osteochondritis, in tuberculosis, mediastinal (case reports), 238
Oxygen, arterial, lack measured by oxygen tension, 315

P^{32}-labeled tubercle bacilli, virulence of, 738
Pancreatitis and other diseases, pleural fluid amylase in, 606
Para-aminosalicylic acid
calcium and calcium-benzoyl, tolerability of, 351
-resistant tubercle bacilli, genetic considerations of mechanisms involved in (Notes), 371

in tuberculosis, pulmonary, with pyrazinamide or isoniazid (Notes), 102
urine test for detection in ambulatory tuberculous patients (Notes), 672
Paratubercle bacilli, skin reaction to products of, 731
Paromomycin in murine leprosy (Notes), 673
PAS. *See* Para-aminosalicylic acid
Pembine type case conference, consecutive, manual for, 258
Pericarditis in tuberculosis, mediastinal (case reports), 238
Peroxidase activities of isoniazid-susceptible and -resistant strains of *M. tuberculosis* (Notes), 669
Physicians on ward, evaluation by patients (Notes), 533
"Plasma factor," circulating levels of, in leukocyte cytolysis in guinea pigs sensitized with *M. tuberculosis* (Notes), 244
Pleura, parietal and visceral, lymphatics as drainage for, 52
Pleural fluid amylase in pancreatitis and other diseases, 606
Pneumococcosis and tuberculosis, 3,3′,5-triiodo-L-thyronine in survival time of mice with, 339
Pneumoconiosis, tuberculosis complicating, chemotherapy in (correspondence), 818
Pneumoliths in silicosis (case reports), 512
Polyoxyethelene ether, failure to protect against tuberculin shock in guinea pigs (Notes), 382
PPD. *See* Tuberculin
Prednisone in effusions, pleural, tuberculous, 307
Protein from paratubercle bacilli, reaction of, and OT, 731
Psychosis, toxic, from isoniazid (case reports), 799
Pyrazinamide in tuberculosis, pulmonary, with isoniazid or PAS (Notes), 102

Rehabilitation
and occupational therapy in tuberculosis hospitals (correspondence), 680
vocational, in tuberculosis (correspondence), 543
Relapse rate in tuberculosis, pulmonary, with and without chemotherapy, 612
Resection
bilateral, pulmonary function in, 468
pulmonary, for drug-resistant cavitary tuberculosis, using ancillary drugs, 780
Resistance, acquired, isoniazid in (Notes), 97
Respiratory function
and altitude, 457
in tuberculosis and other chronic lung diseases (Soviet translation), 142
Ristocetin in murine leprosy (Notes), 673
Roentgenography, survey of community, pulmonary nodules found in, 427

Schistosomiasis, pulmonary, chronic, 119
Serology in relationship of modified sheep and human erythrocytes, 622
Serum
antimycobacterial, antigenicity of, 631
protein electrophoretic pattern and Middlebrook-Dubos titer in BCG-vaccinated tuberculous children (Notes), 522
Silicosis, pneumoliths in (case reports), 512
Skin reaction to products of paratubercle bacilli, 731
Spirometry in maximal breathing capacity, compared with Douglas Bag measurement (Notes), 253
Stenosis, mitral, pulmonary function studies in, 265
Strains, atypical, growth rates of, in biochemical studies (Notes), 94
Streptovaricin in murine leprosy (Notes), 673
Surgery
of blebs, subpleural, 577
reporting of (correspondence), 679
Sweden, BCG vaccination in (correspondence), 678

Test(s)
niacin
in differentiation of tubercle bacilli (Notes), 810
in distinguishing mycobacteria (Notes), 663
of respiratory function, 457
urine, for detecting PAS in ambulatory tuberculous patients (Notes), 672
Thoracoplasty, pre-resection and post-resection, in tuberculosis, 204
Thyroid in tuberculosis, native resistance to, hyperthyroidism in, 152
hypothyroidism in, 180
Tissue culture studies on resistance in tuberculosis, 221
Trachea, fenestration of, evolution and early results of, 773
Tranquilizer(s), effect on activity of ambulatory tuberculous patients (Notes), 531
Transaminase, glutamic oxalacetic and pyruvic, in tuberculosis, pulmonary (Notes), 251
Treatment failures (correspondence), 105
3,3′,5-Triiodo-L-thyronine in tuberculosis and pneumococcosis, survival time of mice with, 339
Triton WR 1339. *See* Polyoxyethelene ether
Tubercle bacillus(i)
differentiation of human from atypical acid-fast (Notes), 810
P^{32}-labeled, virulence of, 738
PAS-resistant, genetic considerations of mechanisms involved in (Notes), 371

in pulmonary lesions, isoniazid effect on growth of (Notes), 518
in tuberculosis, primary, late discharge of, 31
Tuberculin
intracutaneous reaction to, topical hydrocortisone acetate ointment at site of (Notes), 666
OT
and paratubercle bacilli products, skin reaction to, 731
-sensitized sheep and trypsinized human erythrocytes, serologic relation of, 622
PPD and other antigens prepared from atypical acid-fast bacilli and *Nocardia asteroides*, 284
sensitivity and chemotherapy in rabbits, 329
shock, failure of polyoxyethelene ether to protect against, in guinea pigs (Notes), 382
test in case finding in a general hospital, pilot study (Notes), 378
Tuberculosis
alcoholics with, before and during hospitalization (editorials), 659
ambulatory patients with, urine test for detection of PAS in (Notes), 672
bacteriologic media, elimination of precleaning cage-laid hens' eggs in preparation of egg fluid for (Notes), 677
cavitary, drug-resistant, pulmonary resection of, using ancillary drugs, 780
changes as seen by a pathologist (American Trudeau Society conference paper), 684
chemotherapy, 492
complicating pneumoconiosis, chemotherapy in (correspondence), 818
elimination of, as public health problem (American Trudeau Society conference paper), 690
experimental, arrested, isoniazid in (Notes), 246
hospitals, rehabilitation and occupational therapy in (correspondence), 680
in humans
alpha-ethyl-thioisonicotinamide in antituberculous effectiveness of, 6
kanamycin in, 72
natural history of, 19
mediastinal, manifested by pericarditis, osteochondritis, and bronchoesophageal fistula (case reports), 238
in medical students at University of Maryland, 746
nonreactive (case reports), 362
patients, hospitalized, adjustment on different wards, 273
and pneumococcosis, 3,3′,5-triiodo-L-thyronine in survival time of mice with, 339
primary
in children
segmental atelectasis in, 597
segmental lesions in, 756
tubercle bacilli in, late discharge of, 31
pulmonary
bilateral resection for, lung function in, 468
with carcinoma, pulmonary, primary, 134
cycloserine and viomycin in (Notes), 90
isoniazid and cycloserine in (Notes), 87
lung function in, 474
preresection drug therapy in, 41
pyrazinamide with isoniazid or PAS (Notes), 102
relapse rate in, with and without chemotherapy, 612
serum enzymes in (Notes), 251
rehabilitation, vocational, in (correspondence), 543
respiratory function in, and in other chronic lung diseases (Soviet translation), 142
thoracoplasty, pre-resection and post-resection, in, 204
thyroid in native resistance to, 152, 180
tissue culture studies on resistance in, 221
urban reservoirs of (American Trudeau Society conference paper), 687
Tween-albumin liquid medium in differentiation of tubercle bacilli (Notes), 810

United States, Maryland, University of, tuberculosis in medical students at, 746
U. S. S. R.
translation of review from
Puzik and Uvarova, 497
Stepanyan, 142

Vaccination, BCG, in Sweden (correspondence), 678
Vaccine. *See also* BCG
Vaccine(s) from gamma-irradiated *M. tuberculosis* and *Brucella suis* (Notes), 374
Ventilation, helium-dilution method in study of, 450
Viomycin and cycloserine in tuberculosis, pulmonary (Notes), 90

LIST OF JOURNALS ABSTRACTED

A.M.A. American Journal of Diseases of Children
A.M.A. Archives of Industrial Health
A.M.A. Archives of Internal Medicine
A.M.A. Archives of Pathology
A.M.A. Archives of Surgery
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Antibiotics and Chemotherapy
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Archiwum Immunologii i Terapii Doswiadczalnej
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Arquivos de Tisiologia
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Fortschritte auf dem Gebiete der Röntgenstrahlen vereinigt mit Röntgenpraxis
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Giornale italiano della tuberculosi
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Khirurgija
Klinische Wochenschrift
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Lancet
Laval médical
Lékařské Obzor
Lille Medicale
Lotta contro la tubercolosi
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Medicina del lavoro
Medicine
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Microbiologia, Parazitologia, Epidemiologia
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Proceedings of the Staff Meetings of the Mayo Clinic
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Surgery
Surgery, Gynecology and Obstetrics

Thorax
Tórax
Tubercle
Tuberculology
Tuberculoza
Tuberculozis
Tuberkulosearzt

Wiener klinische Wochenschrift
Wiener medizinische Wochenschrift
World Health Organization Newsletter
Yale Journal of Biology and Medicine
Zeitschrift für Tuberkulose
Zentralblatt für die gesamte Tuberkulose-forschung

INDEX OF ABSTRACTS

AUTHORS

Adam, M., 712
Adams, W. E., 709
Adler, D., 711
Adler, J., 405
Adler, R. H., 4
Affeldt, J. E., 30
Agazia, L., 560
Ahn, C. H., 24
Alencar, A. de, 573
Alexander, J. K., 421
Algeorge, G., 570
Aliperta, A., 560
Allen, A. R., 40
Allen, J. H., Jr., 415
Alley, R. D., 18
Almeida, A. S. de, 561
Altmann, V., 558
Altonyan, R. E. C., 700
Alves, M. De Sousa, 4
Alvik, I., 408
Anastasatu, C., 24
Andrade, L. de, 574
Andrews, N. C., 48
Angeli, F., 60
Angeloni, J. M., 707
Anscombe, A. R., 58
Aoyama, Y., 403
Apthorp, G. H., 723
Arany, L. S., 18
Arbman, B., 8
Arhiri, M., 570
Arkhipova, O. P., 30
Armen, R. N., 37
Artigas, G. V., 561
Artusio, J., Jr., 708
Asp, K., 12
Attinger, E., 30
Aufses, A. H., 17

Baba, K., 407
Babcock, G. C., 418
Babolini, G., 560
Bacchus, H., 401
Baicioanu, S., 570
Bailey, D. A., 32
Bailey, K. V., 50
Ball, G. D., 423
Balogh, A., 26
Bamforth, B. J., 19, 20
Ban, K., 701
Bancale, A., 703
Barber, L. M., 561
Barhad, B., 38
Bariéty, M., 40
Barlocher, P., 705
Barnard, P. J., 575
Barnes, W. H., 61
Barois, A., 11
Barrett, R. J., 57, 708
Barrie, J., 712
Bartman, K., 420
Bastarrachea, F., 424
Bates, D. V., 723
Bauer, A. J., 8
Bauer, H. J., 567
Baur, P., 22
Bech, A. O., 552
Bedell, G. N., 58
Beduschi, A., 561
Beerman, H., 38
Behrens, W., 2
Bellville, J., 59
Bentzon, M. W., 423
Berfenstam, R., 729
Berger, M., 402
Bergman, F., 2
Bergoqvist, S., 575
Bergsmann, O., 405
Bernard, E., 11, 22
Bernasconi, C., 559
Bernatz, P. E., 710
Bernstein, W. H., 723
Berteaux, S., 720
Bertheau, M., 570
Bettega, J. E., 561
Bettega, J. L., 561
Beuchner, H. A., 414
Bignamini, A., 52
Björk, V. O., 17
Blahník, Z., 576
Blakemore, W. S., 409
Blasi, A., 560
Blasi, E., 566
Blazsik, C., 554
Blitz, O., 414
Bloch, R. G., 412
Blomquist, E., 21
Bocconi, V. Lamberti, 553
Boche, R. D., 32
Bochinski, K., 3
Boda, D., 572
Bodkin, P. E., 702
Bogardus, G. M., 29
Bojalil, L. F., 424

Boley, J. O., 411
Bonard, E. C., 7
Bond, J. O., 414
Bönicke, R., 60
Bonneau, M., 408
Bonniot, A., 712
Bonte, F. J., 556
Borrie, J., 401
Böszörményi, M., 559
Bottero, A., 570
Boucher, C., 412
Bouhuys, A., 727
Bourgeois, P., 27
Boyden, E. A., 2
Bøyum, S., 558
Brasca, C., 58
Braun, D., 23
Breslaw, L., 2
Bretey, J., 422, 575
Brill, A., 570
Briscoe, W. A., 722
Brown, T., 6
Bruce, R. A., 716
Brunner, A., 34
Brünner, S. A., 555
Bruwer, A., 417
Buchanan, J., 722
Buchanan, W. W., 722
Buchberger, R., 400
Buebendorf, D. P., 414
Bugyi, B., 23
Buhlmann, A., 705
Bull, A. B., 725
Bumbăcescu, N., 718
Bungetianu, Gh., 570, 571
Burford, T. H., 409
Burkart, F., 23
Burnside, A. F., Jr., 710
Burrows, B., 709
Buscarini, L., 559
Butnaru, D., 570
Buxton, R. St. J., 58
Buzescu, M., 570

Cabiran, L. R., 703
Cabrera, S. Zeron, 9
Cadore, F. de, 574
Caiafa, L., 703
Caldwell, D. M., 704
Callahan, J., 417
Cals, Mme S., 27
Canepari, C., 568
Canepari, L., 568
Canetti, G., 26, 575
Cantoni, E., 556
Capone, M., 38
Carey, D. E., 37
Carey, G. C. R., 421
Carey, J. M., 41
Carr, D., 46
Carson, T. E., 414
Cass, L. J., 56
Castellanos, A., 566
Catena, E., 560, 566
Cau, G., 52
Caughey, J. E., 401
Caulet, T. R., 13
Cavin, E., 5
Cazzaniga, G., 553
Center, S., 409
Cerbón, J., 424
Čermák, M., 562
Chace, J. F., 558
Chambatte, Ch., 42
Chamberlain, J. M., 18
Chang, J. C., 11
Chapman, J. S., 707
Chatterjee, S. S., 420
Chen, A., 58
Chenebault, J., 556
Chesnic, J. J., 711
Chigier, E., 59
Choubrac, P., 40
Christie, A., 704
Christoforidis, A., 553
Chu, T. Y., 11
Cicero, R., 16
Clagett, O. T., 710
Claiciu, I., 571
Clarkson, B., 34
Clavel, Mlle S., 27
Cochran, J. B., 714
Cohen, G., 701
Cohen, R., 53
Collier, C. R , 30
Colombi, G. C., 556, 570, 707
Coman, N., 24
Comeau, M., 39
Concina, E., 41
Conn, H. O., 552
Connolly, C., 20
Connolly, J. E., 18
Cooper, N. R., 414
Cope, C., 20
Corbetta, F., 559
Correll, N. O., Jr., 576
Corriol, R., 563
Costa, G., 706, 721
Coster, A. De, 726
Cotter, D. L., 569
Crane, J., 724
Cranny, R. L., 44
Creech, O., 4, 712
Creedon, F. T., 32

Crepy, C., 408
Crofts, N. F., 703
Croizat, P., 402
Crowley, R. T., 708
Crozafon, C., 10
Crump, C. H., 59
Cullell, R., 573
Cummings, M. M., 562
Cunningham, G. J., 560
Curcho, M. de la Garza, 16
Curreri, A. R., 708
Cutforth, R. H., 35
Czeredarczuk, O., 556

Dabbs, C. H., 724
Dalet, R., 26
Dallachy, R., 19
Dallorso, E., 721
Danaraj, T. J., 555
Daniello, L., 24
Dargent, M., 402
Das, J. B., 409
Dauer, C. C., 414
Davis, W. N., 6
Daywitt, A. L., 56
de Alencar, A., 573
de Almeida, A. S., 561
de Andrade, L., 574
Deaton, W., Jr., 19
Debray, C., 711
de Cadore, F., 574
Dechaume, M., 408
De Coster, A., 726
Defares, J. G., 422
de Fraciscis, V., 60
Degli Esposti, A., 26
Degna, A. T., 62
Degna, A. Tommasini, 62
Dekornfeld, T. J., 19, 20
de la Garza Curcho, M., 16
Delong, V., 571
del Molina Mateus, J., 2
de Matteis, M., 572
Dennis, E. W., 421
Denolin, H., 726
Deporte, A., 711
De Sousa, M. Alves, 4
Des Prez, R. M., 406
de Romeuf, J., 570
Diaz, R., 24
Diaz Gomez, L., 10
Dickie, H. A., 36
Dieckmann, B., 14
Dimmick, R. L., 23
di Stefano, A., 55
Doig, A. T., 404
Donomae, I., 54
Dorst, J. P., 567
Dowdy, A. H., 566
Dowling, M., Jr., 59
Doyle, L., 41
Drake, C. S., 400
Dreuttel, M., 569
Dron, F., 38
DuBois, A. B., 722
Dubos-Verliere, Mme, 27
Duby, D., 574
Duchon, J., 28
Dunbar, F. P., 414
Duncan, J. T., 46
Dunér, H., 552
Dunn, F. L., 37
Dunner, L., 715
Durkan, T. M., 32
Dvořák, M., 569
Dworak, W., 3
Dwyer, J., 701
Dybicki, J., 3

Ebersole, C., 708
Ebert, R. V., 36
Edge, J. R., 713
Edgren, W., 14
Edlund, T., 729
Edwards, J. E., 61
Edwards, J. R., 421
Edwards, L. B., 716
Eger, E., 727
Ehrner, L., 726
Eiseman, B., 17
Eisenberg, G. M., 420
Elisberg, E., 38
Ellis, F. H., Jr., 50, 61
Ellman, P., 35
Elo, R., 566
Elrick, H., 56
Emerson, G. L., 25
Emmons, C., 553
Emmons, C. W., 50
Engbaek, H., 423
Engel, H. P., 711
Engel, S., 722
Englert, M., 726
Engström, I., 57, 422, 726
Esposti, A. Degli, 26
Evans, J., 416
Evian, N., 9

Fabre, A., 52
Fabre, Ch., 11
Falk, H. L., 32
Fame, F., 13
Fanconi, A., 2
Fantini, A., 35

Fawcett, A. W., 409
Feinsilver, O., 28
Feldman, A., 418
Ferguson, R. H., 404
Ferguson, T. B., 409
Ferlinz, R., 405
Fernandez Luna, D., 552
Ferreira, M. R., 573
Feuereisl, R., 568
Fierer, E. M., 723
Filippi, A., 53
Fillastre, C., 21, 413
Finby, N., 416
Finkelstein, M., 569
Finoelst, M., 712
Fisher, D. L., 561
Fjelde, A., 422, 423
Flamm, H., 424
Flint, F. J., 401
Flippin, H. F., 420
Foa, G., 53
Fock, G., 410
Ford, R. M., 55
Ford, W. B., 561
Foreman, H. M., 569
Foster, J. J., 702
Foti, E., 15
Fouquet, J., 559
Fournier, P., 571
Fox, R. T., 49
Fox, W., 720
Franchini, C., 568
Francis, R. S., 420
Fraser, J. W., 51
Fraser, K., 47
Frederik, W. S., 56
Fréour, P., 22
Frew, H. W. O., 47
Friedel, H., 410
Friedli, H., 12
Fritz, H., 573
Fruhling, L., 5
Fuchs, B., 716
Fukui, A., 425
Funch, R. B., 418

Gale, D., 574
Gale, J. W., 19, 20, 708
Galliová, J., 564
Gangadharam, P. R. J., 56
Garcia, O., 566
Gardiner, M. P., 41
Garrett, R., 418
Garrigau, J., 42
Garza Curcho, M. de la, 16
Gassman, W., 34
Gelperin, A., 563
Georgesco, P., 729
Gerard, F. P., 719
Giachi, R., 42
Giammalvo, J. T., 553
Gilbert, E., 26
Gillespie, R. W., 554
Giraldi, N., 58
Glas, W., 58
Gloor, H. U., 408
Goldstein, M., 30
Golub, M., 569
Gomez, L. Diaz, 10
Gomi, J., 54
Gonzalez, E., 566
Gonzalez, J. E., 16
Gonzalez Ribas, M., 2
Gordon, E. E., 415
Goto, S., 46, 712
Gough, J., 54
Grahl-Madsen, R., 552
Grant, I. W. B., 705
Grant, J. L., 421
Green, H., 48
Green, L., 16
Greene, E. A., 404
Greer, A. E., 41
Gregoire, F., 39
Gregor, W. McL., 47
Grellet, M., 408
Grieco, A., 59, 725
Griggs, D. E., 30
Grindlay, J. H., 50, 61
Grosset, J., 26, 27, 575
Grove-White, R. J., 54
Grumbach, F., 28
Grumbach, Mlle, 559
Grumbach, Mme F., 27
Guidi, V., 560
Guimaraes, U. Pinheiro, 561
Gupta, S. K., 420
Gupton, E. D., 418
Guy, L. R., 707

Haapanen, J., 9
Hackney, J. D., 30
Haeckel, H., 15
Haggerty, R. J., 403
Halde, C., 715
Hall, A., 36
Hall, J. R., 46
Hall, K., 711
Hamelmann, H., 710
Hamilton, L. C., 554
Hamilton, W., 727
Hammond, D., 724
Hanayama, R., 403
Hanson, A., 35

Harding, H. E., 404
Hardouin, J. P., 711
Hardy, A. V., 414
Hardy, R., 715
Harjola, P., 8
Härö, A. S., 412, 413
Harrell, E. R., 53
Harrigan, P. J., 725
Harrison, C. V., 31
Harrison, R. W., 709
Hart, P. D'Arcy, 61
Harte, M. S., 17
Hartley, L. J., 8
Hartroft, W. S., 6
Hatch, M. T., 23
Hausmann, P. F., 2
Hecht, H. H., 401
Heesen, W., 405
Heiken, C. A., 56
Heimann, Mlle, 559
Heine, F., 403, 418
Heiskala, H., 21
Hellman, L. P., 558
Helm, W. H., 709
Helms, P., 413
Helms, S., 413
Hemingway, A., 421
Hendren, W. H., III, 403
Herman, Z., 405
Herrault, A., 412, 413
Herrault, C. A., 21
Herrmannová, K., 570
Herrnheiser, G., 417
Herscovici, A., 570
Hershberger, R. L., 421
Higgins, I. T. T., 714
Hilbrish, T. F., 553
Hildebrandt, G. S., 409
Hildeen, T., 704
Hilden, T., 6
Hillebrand, H., 418
Hinden, E., 44
Hirayama, M., 425
Hodgson, C., 417
Hofmann, H., 569
Honza, M., 722
Hood, T. R., 404
Horai, Z., 54
Horovitz, N., 9
Hoshika, T., 403
Houde, R., 59
Hrazdira, Č. L., 44
Hrbek, V., 558
Huang, C. S., 44
Huckstep, R. L., 702
Hudgins, P. C., 562
Humbert, W. C., 50
Huppertz, A., 400
Hyman, A. L., 712
Hynková, J., 564

Igarashi, C., 701
Ishida, S., 712
Israel, H. L., 38
Ito, N., 46
Ito, Y., 712
Iwainsky, H., 722
Iwakiri, I., 53
Iwanaga, K., 701

Jackson, A., 404
Jacobs, L. G., 557
Jacobson, H. G., 402
Jacobson, J. W., 32
Jacobson, K. H., 404
James, D. G., 716
Janeček, V., 720
Japanese Association for Tuberculosis, 13
Jarniou, A. P., 42
Jäykkä, S., 723
Jedlička, J., 17
Jefferies, M. B., 414
Jen, C. Y., 44
Jeney, E., 419
Jensen, K. E., 37
Jensik, R. J., 19
Jewett, T. C., 4
Johnson, J., 409
Jones, S. H., 553
Jönsson, R., 727
Jordan, J. W., 400
Josse, J. W., 39
Juselius, H., 4

Kaida, K., 54
Kamen, S. E., 24
Kaňa, P., 564
Kanai, K., 28
Kantor, M., 37
Kara, K., 576
Karlberg, P., 57, 422, 726
Karlhuber, F., 572
Karlson, K. E., 5
Kato, M., 60
Katz, A., F., 10, 29
Kaufman, S., 569
Kaufman, S. A., 567
Kausel, H. W., 18
Kawai, K., 403
Kawaai, N., 54
Kawasaki, M., 701
Keats, T. E., 567
Keil, R., 15
Kelley, V. C., 44

Kellum, H., 712
Kempen, W. V., 406
Kent, E. M., 561
Kerényi, I., 417
Kerley, P., 402
Kesztele, V., 572
Kettner, D., 715
Khait, S. L., 60
Kim, T. C., 24
King, E. J., 31
Kirby, C. K., 409
Kirchhoff, H., 44
Kitahara, T., 53
Kitamoto, O., 54
Kittle, C. F., 411
Kjaer, T., 45
Klassen, K. P., 48
Kleinschmidt, H., 717
Kluge, J., 407
Knox, J., 48
Knutson, D. L., 702
Kobosilová, V., 564
Koch, G., 57
Kohout, M., 571
Komisaruk, R., 57
Komori, Y., 54
Könn, G., 410
Kosinová, M., 557
Kósmiderski, S., 60
Kotin, P., 32
Kovach, R. D., 3
Kovács, K., 5
Kováts, F., Sr., 417
Kraepelian, S., 57, 58, 422, 572, 726
Kraus, P., 568, 572
Krejzková, V., 563
Krepler, P., 424
Křivinka, R., 564
Krogsgaard, A. R., 6, 704
Kröker, P., 419
Kromer, J. P., 9
Kropaczek, Z., 43
Krulík, R., 571
Kubašta, M., 554
Kuentz, M., 402
Kuklovský, J., 559
Kümmerle, F., 710
Kulczycki, L. L., 36
Kuno, S., 487
Kuo, T. L., 11
Kuruez, J., 576
Kusano, N., 403
Kutz, E. R., 417
Kyle, L. H., 3

Laconi, A., 418
Laforet, E. G., 408
Lambert, R., 56
Lamberti Bocconi, V., 553
Lambros, T. A., 407
Land, E. K., 566
Lang, V., 553
Langerová, M., 706
Langston, H. T., 410, 576
Lanier, R. R., 418
Lapras, A., 726
Large, S. E., 45
Lasry, J. E., 18
Laughlin, J. S., 418
Lazarou, P., 13
Lee, L., Jr., 58
Lees, W. M., 49
Leggat, P. O., 401
Legters, L. J., 18
Lehto, S., 56
Leib, G. M. P., 6
Leidi, F., 570
Leizerovici, L., 718
Le Melletier, J., 13
Lemon, F. R., 715
Lennel, J., 11
Lenoble, L., 402
Leonardon, P., 26
Lepengle, J., 26
Lepeuple, A., 27
Lerrel, J., 574
Lesorbe, R., 22, 24
Letnansky, K., 400
Levene, G., 567
Levin, B., 403
Levinský, L., 17
Levi-Valensi, A., 26
Lewis, A. G., 414
Lewis, B. M., 57
Lewis, D. O., 568
Li, M. H., 34
Libermann, 559
Libermann, C., 31
Libermann, D., 27
Lin, T. -H., 57
Lincoln, T. A., 418
Linder, E., 2
Lindgren, P., 566
Lisboa, B. P., 60
Lithander, A., 575
Little, R. C., 701
Lloyd, J., 35
Lodwick, G. S., 567
Löfström, B., 18
Loitman, B., 37
Lojacono, L., 556, 707
Lomartire, N., 723
Long, E. T., 709
Longin, F., 555

Loosli, C. G., 421
Lorber, J., 567
Lorge, H. J., 42
Lott, J. S., 417
Loubry, P., 412
Louria, D. B., 553
Lucaciu, V., 24
Lucas, B. G., 725
Lueders, H. W., 18
Luhr, J., 552
Lukáči, J., 705
Lull, G. F., Jr., 702
Luna, D. Fernandez, 552
Lund, F., 15
Lundberg, A., 29
Lundin, G., 727
Ľuptáková, H., 718
Luridiana, N., 719
Lutz, A., 28
Lyons, H. A., 719

McAfee, J. G., 566
McCallum, R. I., 6
McClement, J. H., 401
McCullough, N. B., 553
MacDonald, A., 421
McFall, R. A., 566
McGuiness, J. B., 722
Machado, S. M., 561
Mackeprang, B., 423, 424
McLaughlin, A. I. G., 404
McLean, K. H., 35
McLeod, J. A., 52
MacMoran, J. W., 418
McNamara, D. H., 704
McQuiggan, M., 6
Maeda, M., 562, 716
Mael, Mlle, 27
Magistretti, M., 723
Magistro, R., 15
Magnin, F., 574
Málek, I., 575
Malgras, J., 574
Mande, R., 21, 412, 413
Manion, W. C., 411
Marconi, P., 713
Marie, M., 408
Marshall, R., 30, 57, 723
Martin, E., 711
Martin, J., 415
Martin, J. D., 37
Martinotti, G., 42
Martoni, L., 26
Mason, R., 728
Masters, J. H., 5
Mateus, J. del Molina, 2
Maťková, V., 564
Matsaniotis, N., 721
Matsumoto, M., 403
Matsunaga, K., 60
Matsuyama, T., 403
Matteis, M. de, 572
Mattingly, T. W., 411
Matts, S. G. F., 34
Mayer, E., 554
Mayer, J., 574
Mečíř, S., 559
Meiss, J. H., 50
Meister, L., 705
Mellor, W. C., 707
Melrose, A. G., 722
Mendel, D., 723
Menz, R., 556
Merrill, D. L., 561
Messina, G. U., 703
Meurk, M. L., 418
Meurman, O. H., 4
Migueres, J., 26
Miki, K., 60
Millbourn, E. W., 19
Miller, W. F., 3
Miscall, L., 5
Mise, J., 421
Mitchison, D. A., 56, 423
Moeschlin, S., 400
Mohelská, H., 564
Molina Mateus, J. del, 2
Monaco, A., 53
Monk, M. A., 413
Montmorency, F., 58
Moody, J., 5
Moreau, A., 42
Moretti, E., 706, 721
Morgan, W. K. C., 45
Morkovin, D., 418
Morrow, C. S., 37
Mostyn, M., 47
Mowlan, A., 20
Moyeux, M., 27
Moynihan, M. J., 414
Muftic, M. K., 721
Mulhim, R., 20
Mulka-Padhaya, B., 14
Mullaney, P. J., 729
Müller, W., 13
Munkner, T., 719
Murányi, L., 572
Murasawa, K., 558
Murohashi, T., 562, 716
Muth, R. G., 44

Nagashima, K., 712
Nagata, S., 727
Nagelschmidt, G., 31

Naito, M., 54
Nakajima, M., 28
Nakamura, S., 425
Nakamura, T., 712
Nakielny, E., 715
Nassau, E., 560
Nasta, M., 570, 729
Nègre, L., 422
Negri, M., 707
Negulescu, V., 570
Nelson, S. W., 553
Neradová, O., 558
Neumann, G., 406
Neville, J. F., Jr., 561
Newell, D. J., 6
Newstrand, D., 715
Ng., J., 23
Ngai, S., 48
Nichols, G., Jr., 57
Nicholson, O. R., 408
Nickell, W. K., 411
Niedner, K., 15
Ninomiya, S., 403
Nisell, O., 726
Noe, F. E., 57
Noehren, T. H., 18
Nohl, H. C., 728
Norris, F., Jr., 711
Norris, S., 711
Noufflard, H., 720

Oberman, J., 26
Ochsner, A., 20
Ochsner, S., 20
Oderr, C. P., 417
Ogawa, Y., 425
O'Hagan-Ward, F. A., 702
Ohara, I., 416
Oka, S., 54
Olansky, S., 50
Oliver, T. K., Jr., 726
O'Loughlin, B. J., 566
Ontario Tuberculosis Association, Medical Section, Committee on Radiation, 25
Oppenheimer, M. J., 418
Oram, S., 35
Ordman, D., 714
Organick, A., 406
Orlandi, O., 41
O'Rourke, P. V., 57
Osada, R., 403
Oscarsson, P. N., 23
Oshima, K., 425
Ošťádalová, B., 564, 565
Oudet, P., 9
Oury, M., 22, 24
Ouvarova, O. A., 30
Overton, R. C., 4
Owens, D., 722

Pacheco, C. R., 16
Packalén, T., 575
Pacy, H., 415
Palatresi, R., 568
Palmer, C. E., 716
Palva, T., 405, 411, 566
Pannesco, E., 729
Paolini, P., 35
Papillon, J., 402
Paris, J., 404
Parish, C., 407
Parker, B. M., 4
Parker, R. G., 46
Parmley, L. F., 411
Parrella, M., 560, 713
Parry, E. H. O., 406
Pasargiklian, M., 58
Pastega, G., 560
Pastras, T., 38
Paterson, A. B., 728
Pătiălă, J., 413
Patterson, D. S. P., 728
Paulson, D. L., 701
Payen, J., 408
Pearson, J. D., 703
Peck, A. S., 18
Pecora, D. V., 45
Peirce, E. C., II, 724
Peirone, E., 723
Pelikán, V., 568
Pepler, W. J., 705
Pepys, J., 716
Pérez-Tamayo, R., 424
Perna, G., 570
Perna, L., 713
Pernis, B., 62
Pernow, B., 552
Pescetti, G., 41, 43
Petersen, O., 719
Peukert, D., 722
Pezza, M., 59, 560
Pfitzer, E. A., 404
Phair, J. J., 421
Phillips, L. A., 566
Pichel, R. R., 3
Pickroth, G., 15
Piéchaud, F., 22
Pierce, J. A., 36
Pieron, R., 11
Pinet, F., 402
Pinheiro Guimaraes, U., 561
Pino, D. M., 20
Pinsky, H. J., 25
Piper, D. W., 49

Pizzolato, P., 417
Poetter, H. W., 701
Poker, N., 416
Pokorný, J., 574
Polák, E., 17
Polánský, F., 718
Polisena, G., 37
Ponthus, P., 402
Popovici, N., 569
Poppel, M. H., 402
Popper, M., 6, 569
Porot, M., 26
Póta, L., 417
Prevatt, A. L., 414
Prévot, A. -R., 574
Prez, R. M. Des, 406
Price, A. V., 722
Procknow, J. J., 421
Prockop, D. J., 34
Proetel, H., 410
Prokopowicz, J., 54

Quilligan, J. J., Jr., 32

Raber, R., 5
Rádl, J., 576
Rainer, W. G., 17
Rankin, J., 36
Rannon, L., 59
Rantz, L. A., 715
Rao, A. Ranga, 12
Rapaport, C., 46
Rappaport, I., 554
Rawson, F. L., 724
Rebora, F., 10
Recant, L., 6
Redlin, A. J., 32
Reemtsma, K., 712
Reid, H. C., 19
Reid, L., 719
Reimann, A., 709
Reindell, H., 13
Reingold, I. M., 705
Renovan, H. -D., 13
Ressl, J., 554
Revill, D., 34
Revol, L., 402
Reynolds, J., 556
Ribas, M. Gonzalez, 2
Rist, N., 27, 559
Rivera, M., 10
Roberto, F., 53
Robicsek, F., 15
Robicsek, L., 15
Robin, E. D., 59
Robinson, L. B., 715
Robinson, R. Q., 37
Robinson, W., 700
Roche, H., 21
Rockoff, S. D., 558
Rodescu, D., 569
Rogers, W. K., 724
Rollier, R., 556
Romeuf, J. de, 570
Roos, B. E., 557
Ross, J. D., 705
Ross, R. R., 721
Rossi, S., 37
Rotem, Y., 59
Roujeau, J., 570
Rowe, G. G., 708
Rowe, R. D., 724
Rowland, H. A. K., 53
Rowlandson, R., 46
Roy, Mme D., 422
Rozner, W., 35
Rubio Palacios, H., 10
Rudberg-Roos, I., 557
Rudel, H., 58
Rudolf, L. E., 49
Ruebsamen, G., 9
Ruggieri, M., 55
Rüll, J., 28
Rulten, R., 423
Rustichelli, V., 37

Sabato, G., 55
Saenz, A., 26
Saenz, A., Jr., 575
Šafařík, L., 571
Saffiotti, U., 62
Saito, T., 701
Saito, Y., 43
Šalanská, V., 574
Saltzman, G. F., 568
Salyer, J. M., 8
Samet, P., 723
Samson, P. C., 561
Samuel, K. C., 408
Sanderud, K., 562
Sanger, P. W., 15
Sanghvi, L. M., 408
Sarot, I. A., 560
Sartorelli, E., 59, 723, 725
Sartory, K., 574
Sasaki, T., 712
Sattler, A., 7, 703
Sayeg, F., 561
Sbertoli, C., 59
Schaub, F., 705
Schepers, G. W. H., 32
Schieppati, E., 49
Schiødt, T., 38
Schmidt, J. G., 32

Schmiedel, A., 420
Schobinger, R., 24
Schoen, I., 705
Schramel, R. J., 712
Schroeder, C., 405
Schultz, J., 60
Schulze, W., 556
Schurch, P., 574
Schürmeier, E., 403
Schwartz, J., 5
Schwartz, P., 40, 706
Schwarz, E., 402
Schweiger, O., 574
Scoditti, G., 55
Scott, A. W., 707
Scotti, P., 725
Seal, R. M. E., 569
Seebohm, P. M., 58
Seelich, F., 400
Segal, M., 30
Selkon, J. B., 423
Šembera, R., 559
Senis, F., 59, 560
Senning, A., 48
Seremetis, M., 560
Sergeant, Y. H., 571
Serisé, M., 22
Shapiro, J. H., 402
Shaw, R. S., 726
Shephard, R. J., 421
Sherman, R. S., 418
Shields, T. W., 49
Shih, M. H., 44
Shimamura, K., 54
Shipley, R. E., 59
Short, E. I., 56
Shubin, H., 56
Shuford, W., 416
Shwachman, H., 36
Sikand, B. K., 12
Silander, T., 410
Silver, C. P., 51
Silwer, H., 23
Šimáně, Z., 572
Simler, M., 5
Simmons, D. H., 421
Simon, G., 719
Simpson, T., 700
Singhaphakdi, O. S., 56
Sirola, R., 4
Sisti, M. A., 55, 703
Skinner, E. F., 46
Skládal, J., 725
Sklenovský, A., 571
Slocum, H., 48
Smart, G. A., 715
Smart, J., 54
Smidt, C. M., 45
Smith, D., 5
Smith, H. Williams, 61
Smith, I. H., 417
Smith, J. G., Jr., 50
Smith, J. R., 4
Smith, M. H. D., 721
Smith, R. A., 552
Sobel, L., 557
Sones, M., 38
Sorensen, H. R., 45
Sousa, M. Alves De, 4
Southren, A. L., 3
Špinková, V., 571
Spitz, H., 405
Spjut, H. J., 409
Spurny, J., 15
Squadrito, G., 38
Srouji, M., 20
Stacey, R. R., 421
Staffieri, P., 37
Staines, E., 11, 29
Šťastná, J., 564
Stauffer, H. M., 418
Steenburg, R. W., 49
Stefano, A. di, 55
Steinberg, I., 5, 7, 403, 416
Stern, W. Z., 402
Steurman, J., 569
Stockdale, C. R., 707
Stonebrink, B., 574
Stranahan, A., 18
Sträuli, P., 714
Street, B. J., 38
Strieder, J. W., 408
Stueck, G. H., Jr., 421
Subbaiah, T. V., 56
Suess, H. O., 26
Šula, L., 25
Šulová, J., 25
Sun, S. T., 34
Sunahara, M., 54
Supramaniam, J. M. J., 54
Suzuki, C., 712
Suzuki, Y., 712
Suzumura, F., 42
Svoboda, J., 563, 713
Svobodová, L., 563, 713
Svrčková, M., 718
Sweetman, W. R., 8
Sýkora, F., 717
Szczubowski, M. J., 56
Szécsey, G., 5

Tacquet, A., 571
Taka, K., 425
Takeuchi, H., 425

Takizawa, K., 712
Tala, P., 4
Tamayo, R., Pérez-, 424
Tamusz, O., 418
Tanabe, T., 46
Tanno, A., 416
Tarnowski, C. E., 23
Taylor, A. J., 576, 703
Tegeris, A., 5
Teixeira, J., 573
Terada, K., 53
Terris, M., 413
Teska, O., 568
Teyssier, L., 559
Thackray, A. C., 47
Thakre, M. W., 420
Thibaudeau, Y., 39
Thibier, R., 26, 27
Thomas, A. M., 404
Thomas, D. E., 38
Thomas, L. B., 553
Thompson, G. R., 53
Thompson, J., 414
Thompson, V. C., 709
Thomson, A. D., 47
Thomson, M. L., 421
Timmes, J. J., 5
Tobrman, B., 715
Todosijczuk, D., 702
Tomasini, M., 725
Tommasini Degna, A., 62
Tomoda, T., 728
Tompkins, R., 724
Tornell, G., 566
Toušek, M., 576
Tozzi, A., 707
Trasko, V. M., 415
Travis, D. M., 59
Trefný, J., 564, 718
Trivedi, S. A., 39
Truan, T., 23
Tschumy, W., 555
Tsenghi, C., 721
Turunen, M., 8
Tuttle, W. M., 57, 708
Tuzi, T., 59
Twelfth National Congress of Tuberculosis, Montpellier, 729

Uchida, S., 403
Uchiyama, H., 562, 716
Ulrich, A., 557
Unteanu, Gh., 6
Urban, J., 718
Urbanský, A., 705

Vainio, S., 14
Van der Hoeven, L., 423
Van Hazel, W., 19
Van Houten, R. J., 20
v. d. Lühe, I., 15
Van der Sar, A., 423
Varpela, E., 413
Vartio, T., 43
Vašečka, I., 556
Vaz, A., 573
Veall, N., 703
Vergani, F., 58
Vesterdal, J., 719
Viklický, J., 557
Vimtrup, B. J., 6, 704
Vivien, J. N., 26, 27
Vlad, A., 38
Voisin, C., 571
Voisin, R., 563

Waingortin, E., 42
Waisbren, B. A., 415
Wald, G., 59
Walker, J. M., 39
Wallenstein, S., 59
Wallgren, S., 563
Walter, J. B., 560
Walton, E. W., 39
Wan, T. H., 44
Wang, R. I. H., 59
Ware, G. W., 36
Warnery, M., 563
Warrack, A. J. N., 401
Warren, R., 49
Warter, J., 5
Weens, H., 416
Weingärtner, L., 25
Weisert, O., 420
Weiss, A. G., 9
Weiss, D. L., 556
Weiss, W., 420
Welch, R. I. H., 705
Welsh, J. D., 408
Wengler, G., 726
Werner, E., 7
Whaley, R. D., 59
Wheeler, W. E., 726
Whitcher, H. W., 725
White, M., 18
Wilcox, K. R., 415
Wilkins, E. W., Jr., 25
Williams, H., 54
Wilson, F. F., 401
Wilson, J. A., 702
Wilson, J. L., 20
Wilson, J. T., 408
Wilson, R. E., 49
Wilson, T. M., 41
Wilson, W. R., 58
Winslow, W., 37

Witz, J. P., 9
Witz, Mme, 28
Wolf, A., 6
Wolfová, B., 575
Woodruff, P. S., 565
Woolley, P. B., 700
Wright, E. C., 728
Wurm, K., 13
Wyman, S. M., 25
Wynder, E. L., 715

Xalabarder, C., 573

Yachno, M. A., 60
Yamakami, J., 712
Yamaki, I., 407
Yamamoto, T., 419
Yamamura, Y., 61
Yanagisawa, K., 28
Yankee, R. A., 414
Yasukawa, T., 700
Yee, J., 561
Yefimova, V. E., 60
Yevich, P. P., 404
Yoganathan, M., 31
Yokoyama, K., 53
Young, B. R., 418
Young, F. H., 711
Young, W. P., 708

Zaccone, G., 418
Zacks, S. I., 39
Zaidi, S., 31
Zajíček, K., 557
Žák, F., 17
Zakstelskaya, L. Y., 60
Zděnková, B., 557
Zederfeldt, B., 18
Zemánek, J., 558
Zenker, H., 707
Zeron Cabrera, S., 9
Zettergren, L., 729
Zhadanov, V. M., 60
Zimmermann, B., 20
Zimmermann, W., 405
Ziskind, J., 417
Zocchi, S., 706
Zsolnai, T., 419

Abdomen, surgery, effect on lung capacity, total, 58
Abscess
  cold, paravertebral, secondarily infected, discharging, excision in, 407
  of liver and fistulas, pulmonary, arteriovenous, 5
  of lung
    complicating cavity, tuberculous, open, healed, 2
    and pneumonia in influenza, 707
Achalasia of esophagus, surgery in, 708
Acid(s), nitric, red fuming, acute toxicity of, 404
Acidosis, respiratory, acute, serial changes in tissue carbon dioxide content during, 57
ACTH. *See* Hormones, corticotropin
Actinomycin C, tuberculostatic effect of, 25
Adenoma. *See* Tumor(s)
Adenopathy
  mediastinal, in tuberculosis, etiologic diagnosis of, 42
  tracheobronchial, tuberculous, in adults, 13
Adenosine, deaminase activity in blood of cancer patients, 400
Adenovirus
  in pneumonia, infantile, fatal, 403
  intermediate type, isolation from fatal case of infantile pneumonia, 403
Adrenal secretion in tuberculous patients on corticotropin, 570
Adrenocortical hormones. *See* Hormones
Aerosols, particle-sizing method for, and fine powders, 23
Agammaglobulinemia, roentgenography in, 415
Age and respiratory function in normal men at work, 59
Aged
  emphysema in, 554
  pleurisy with effusion in, 557
Airway conductance, airway resistance, and lung volume, relationship as to age and body size, 722
Airway resistance, airway conductance, and lung volume relationship as to age and body size, 722
Alcoholism and tuberculosis morbidity, 563
Allergy
  infra-, demonstrated by total bacterial cells and antituberculous resistance due to BCG, 422
  respiratory from cereal grain dust, in South Africa, 714
  tuberculin from BCG and virulent tubercle bacilli, 413
Alpha-ethyl-thioisonicotinamide in tubercle bacilli, isoniazid-resistant, 27
Altitude and respiratory pathology, 726
Alveolar
  air, fractional analysis of, in measuring diffusing capacity of lungs for carbon monoxide, 30, 57
  gas tensions in physiologic sleep in normal subjects, 59
  ventilation
    and physiologic dead space studies, 726
    and respiratory dead space, 725
p-Aminosalol in tuberculosis, pulmonary, 26
Amyloidosis, tumor-forming, of lung, 2
Analgesia, ether, inspired concentrations, flammability, and levels in arterial blood, 708
Anastomosis, vena cava-pulmonary artery, for lung vascularization, 15
Anatomy, studies of, compared with roentgenography in tuberculosis mortality, 24
Anemia, aregenerative, and thymoma, benign, 34
Anesthesia
  in resection, pulmonary, 20
  in thoracic surgery, 20
Aneurysm
  congenital, of coronary artery, with arteriovenous fistula, 719
  pulmonary, arteriovenous, in telangiectasis, hereditary, 34
  of thoracic aorta, traumatic, chronic, acute, roentgen diagnosis in, 416
Angiography. *See* Roentgenography
Anovaria causing nanosomia and geroderma, 17
Antibody(ies)
  response of tuberculous guinea pig to nontuberculous antigens, 575
  tuberculous, in Boeck's disease, 13
Antimicrobials. *See also* Chemotherapy
Antimicrobials
  compound B-663, activity of, 720
  and corticotropin in tuberculous peritonitis, 568
  in fatal fungal infection in sarcoidosis, 7
  intracavitary injections of, 720
  new
    meleinic acid hydrazid and maleinic acid phenylhydrazid, 419
    reported at International Union Against Tuberculosis, Paris, July, 1958, 419
  in pleurisy, tuberculous, exudative, with adrenocortical hormones, 53
  with prednisolone, in silicosis, 11
  prolonged, in tuberculosis, pulmonary, far advanced, 9
  in tubercle bacilli, *in vitro* phagocytosis of, 60
Antitussives
  non-narcotic, cough treated by, 39
  sustained release, clinical evaluation of, 56

Aorta
ascending, tuberculous perforation of, 9
coarctation of, resection for, infarction of ileum after, 19
thoracic
aneurysm, traumatic, chronic, acute, of, roentgen diagnosis in, 416
post-traumatic rupture of, clinical management of, 17
tortuous, abnormal mediastinal shadows from, 416
Aortic graft in superior vena caval syndrome, 16
Arterial distribution to pulmonary lobe, right upper, 57
Artery(ies)
anomalous, in intralobar bronchopulmonary sequestration, angiography of, 719
blood levels in with ether analgesia, 708
coronary
abnormal origin of, from pulmonary artery, 552
aneurysm, congenital, of, with arteriovenous fistula, 719
disease of, and smoking, 715
pulmonary
abnormal origin of coronary arteries from, 552
assessment of muscular hypertrophy and endarteritis obliterans in, 575
subclavian, roentgenographic studies of, 416
vena cava—pulmonary, anastomosis of, for lung vascularization, 15
Asbestos miners, lung cancer in, 23
Asian patients, cycloserine in tuberculosis, pulmonary, in, 54
Aspergilloma
pulmonary, 2
so-called, 34
Aspergillosis. *See* Mycoses
Asterixis in pulmonary disease, chronic, 552
Asthma
bronchial
atelectasis in, in childhood, 552
histamine, urinary excretion of, in, 552
provocation tests in, 57
children with, respiratory studies in, 726
symptom-free
children with, lung volume relationships in, 422
effect of bronchodilator drugs on lung volumes of children, 572
Atelectasis in asthma, bronchial, in childhood, 552
Atresia, esophageal, and distant membranous obstruction of esophagus, 4
Australia
South, recent trends in tuberculosis, 565
tuberculosis in, 21
Ayre's T-piece, modified, in positive-negative pressure ventilation, 727
Azorhodanid. *See* 2,4 Diaminoazobenzol-monorhodanid
Azygos lobe of left lung, 34

B-663, antituberculous activity of, 720
Bacillus(i)
acid-fast
atypical, pulmonary diseases from, 414
isolated from lymph nodes and spleen of cattle, 61
unusual, causing systemic disease and death in a child, 423
tubercle. *See* Tubercle bacillus(i)
Bacillus bronchisepticus as cause of human diseases, 424
Bacteria, the starting point of, 573
Bagassosis, 414
Baritosis, 400
Bats in histoplasmosis, 50
BCG
antituberculous resistance due to, and total bacterial cells, infra-allergy demonstrated by, 422
-induced immunity in guinea pigs, isoniazid in, 575
isoniazid-resistant, isolation and maintenance of, 727
lymphadenitis, purulent, after, 21
test in practice, 713
tuberculin allergy from, 413
-vaccinated children, bone and joint tuberculosis in, 713
vaccination
intracutaneous and peroral, of newborns, 565
lymphadenitis, suppurative, with caseous tubercle formation after, 44
role of, 713
tuberculin allergy in guinea pigs after, and subsequent infection with virulent tubercle bacilli, 575
vaccine, grown in Dubos medium, first experiences of vaccination with, 564
-vaccinees, tuberculosis in, 21
Bee propolis, antituberculous properties of, 568
Beryllium poisoning, chronic, 400
Beta-diethylaminoethyl-fencholate in histoplasmosis, pulmonary, acute, disseminated, and cortisone, 5
Biopsy
bronchial, in tuberculous bronchitis, 405
lymph node,
prescalene, 408
scalene, nonpalpable, diagnostic value of, in chest diseases, 49

needle
of parietal pleura, 408
pleural, new, preliminary study, 20
pericardial, with Vim-Silverman needle, 408
pulmonary, practical method for, 553
scalene node, in intrathoracic disease, 728
Bissinosis, 2
Bland-White-Garland syndrome, angiocardiography in, 566
Blastomycosis. *See* Mycoses
Blood. *See also* Plasma
arterial levels in, with ether analgesia, 708
gases, partial pressure of, new method for measurement of, 572
iodine concentration after bronchography, 566
pH of, in physiologic sleep in normal subjects, 59
serum
isoniazid, active, in, 27
effect of PAS adjunct to isoniazid on, 26
volume, capillary, pulmonary, measurement of, 57
Boeck's disease. *See also* Sarcoidosis
tuberculous antibodies in, 13
Bone
tuberculosis, pelvic, and hip joint involvement, 15
and joint tuberculosis, 14
Bordetella bronchiseptica. *See Bacillus bronchisepticus*
Bronchial stump, carcinoma of, recurrent, radiation therapy in, 25
Bronchiectasis
clinical studies of, 700
and neuropathy, peripheral, 401
resection in, long-term results of, 709
reversible, 553
surgical treatment of, 44
in tuberculosis, pulmonary, bronchography and anatomy for, 410
tumor-like formation frequence in, 560
Bronchiole(i) and bronchi, 722
Bronchiolitis, chronic, obliterating, 2
Bronchitis
in agricultural community in Dumfriesshire, 714
chronic
bronchodilators in, 700
and emphysema, 700
reduction of sputum viscosity in, 700
tetracycline, long-term, in, 722
trypsin, intramuscular, in, 701
tuberculous, bronchial biopsies in, 405
Bronchodilators
in bronchitis, chronic, 700
effect of, on lung volumes in symptom-free asthmatic children, 572
by inhalation, comparative effect of, 701
Bronchography
for bronchiectasis in tuberculosis, pulmonary, 410
in hydatid cyst of lung after internal suture of pericyst, 20
in tuberculosis, pulmonary, 52
and bronchial disease, 52
Bronchopneumopathies
from anatomic malformation, 560
the vascular system in, 566
Bronchopulmonary disease, chronic, physical therapy in, 3
Bronchoscopy
in children, 410
promethazine hydrochloride in, 20
in tuberculosis, pulmonary, 12
in tuberculous lymph node perforation into bronchial tree of adult, 405
Bronchospirometry in pulmonary lesions, monolateral, 58
Bronchus
anomaly, unusual, of, 35
and bronchioli, 722
carcinoids of, clinical findings in, 400
carcinoma of
adenosine deaminase activity in plasma of patients with, 400
results in treatment of, 409
diseases of, in tuberculosis, pulmonary, and bronchography, 52
and esophagus, fistulas of, 402
kinked, simulating bronchial stenosis, 17
main-stem, sleeve resection, in bronchial adenoma, 18
pathology of, correlated with tuberculosis, primary, chronic, and evolutive, 41
photography of, with simple apparatus, 411
tracheal
additional, 3
emphysema localized, from, 555
tree of, tuberculous lymph node perforation into, bronchoscopy in, 405

Cadmium reaction. *See* Tests
Calcium
B-PAS in tuberculosis, pulmonary, drug resistance to, 568
fluoride, tissue reactions produced by, in rat lungs, 31
Cancer. *See also* Carcinoma
cells, human, in tissue culture, effect on tubercle bacilli, 422
of lung
in asbestos miners, 23
cobalt-60 intensive teletherapy in, 417
in Denmark, 45
diagnosis of, 702
in females, 714
primary
diagnosis of, transverse hilar measurements in, 567
and tuberculosis, pulmonary, 558

unusual roentgenographic and clinical findings in, 553
and smoking, 715
Candida
fungi, isolation of, from tuberculous patients, 407
in tuberculosis, pulmonary, 706
Capillary(ies)
blood volume, pulmonary, measurement of, 57
erection of, and lungs, fetal and neonatal, structural appearance of, 723
Carbon dioxide
analyzer in ventilatory failure, rapid diagnosis of, 30
respiratory response to noscapine and codeine in, 59
tension, mean venous blood, determination of from exponential carbon dioxide rise during rebreathing, 422
Carbon monoxide
diffusing capacity of lungs for, methods of measuring, 57
poisoning, acute, effect on work capacity, 723
Carcinogenesis, new approach to, 728
Carcinoid. *See* Tumor(s)
Carcinoma. *See also* Cancer
bronchial
in young adults, 45
co-existing with sarcoidosis, 35
with osteoarthropathy, pulmonary, hypertrophic, vagotomy in, 702
of bronchial stump, recurrent, radiation therapy in, 25
bronchogenic
adenosine deaminase activity in plasma of patients with, 400
cavity formation in, 701
and Cushing's syndrome, 3
early detection and treatment of, 701
results in treatment of, 409
in tuberculosis, pulmonary, 41
of esophagus
cobalt-60 beam therapy in, 417
simplified cytodiagnosis in, 711
pulmonary
cytology in diagnosis of, 4
metastatic, cor pulmonale, subacute, from, 553
nonresectable, angiocardiography in, 25
"radical" pneumonectomy in, 409
and tuberculosis, pulmonary, 706
Carcinoids. *See* Tumor(s)
Cardiac cycle, normal, analytic roentgen kymography of, 418
Cardiopulmonary disease, breathing mechanics in, 30
Cardiopulmonary function, epinephrine (racemic) inhalation, effects on in normal and chronic pulmonary emphysematous subjects, 421
Catalase activity of mycobacteria, 574
Cattle, isolation of *M. johnei* from lymph nodes and spleen of, 61
Cavernostomy, open, for superior segmental cavity in a single remaining lung, 560
Cavity(ies)
formation of, in carcinoma, bronchogenic, 701
local chemotherapy in, 569
pneumolysis, management of, 409
segmental, superior, cavernostomy, open, for, in single remaining lung, 560
tuberculous
endobronchial, controlled, antimicrobial treatment of, 42
experimental
by air-borne infection, in rabbit lung, 425
and encapsulated caseous foci, 425
open healing of
in conservatively treated patients, 41
lung abscess complicating, 2
pulmonary, seen roentgenographically and on resected specimens, 24
walls of, histology in, 407
Cell(s)
mammalian, and mycobacteria, in tissue culture, 423
plasma, tumor, of trachea, 705
pulmonary, mast, in experimental silicosis in rat, 62
Ceramic industry, fireproof, silicosis in, 23
Cerase as antituberculous agent, 721
Cetavlon®. *See* Cetyltrimethylammonium bromide
Cetyltrimethylammonium bromide in tubercle bacilli cultivation, with pancreatin, 12
Chemotherapy. *See also* Antimicrobials
intravenous, in tuberculosis, pulmonary, 569
local, for cavity treatment, 569
long-term, of tuberculosis, pulmonary, silicosis-complicated, 419
pathology and bacteriology of resected tuberculous lung lesions after, 569
and cortisone in pleurisy, tuberculous, 53
prophylactic, in tuberculosis, experimental, 570
recurrences after, in tuberculosis in children, 25
in tuberculosis
fatal complications, 569
in infants, 55
pulmonary, for outpatients, 718
tuberculous lesion deterioration during, prednisone in, 722
Chest, barrel, with senile lung and obstructive pulmonary emphysema, 36
Chloroform, inhalers evaluated for, 48
Cholelithiasis with hiatal hernia and diverticulosis coli, 702
"Chronic mountain sickness" in U. S., 401
Climate and cough relationship in a smogless New South Wales coastal district, 415
Coal miners, pneumoconiosis of, in Northumbrian collieries, 6

Cobalt-60
beam therapy in carcinoma of esophagus, 417
teletherapy, intensive, in lung cancer, 417
Coccidioides immitis, nitrofurantoin in, 53
Codeine in respiratory response to carbon dioxide, 59
Cor pulmonale
in cardiopulmonary reserve reduction after pulmonary resection, 709
subacute, from metastatic carcinoma of lung, 553
in tuberculosis, pulmonary, 42
Cortical function, influence of isoniazid on, 571
Corticosteroids. *See* Hormones
Corticotropin. *See* Hormones
Cortisone. *See* Hormones
Cough
antitussive, non-narcotic, in, 39
and climate relationship in a smogless New South Wales coastal district, 415
Cryptococcosis. *See* Mycoses
Cushing's syndrome and carcinoma, bronchogenic, 3
Cycloserine
hemorrhage during treatment with, 570
in Löwenstein-Jensen medium, tubercle bacilli resistance determination in, 420
resistance to, 570
in tuberculosis
neuropsychiatric accidents from, 26
pulmonary, 10, 570, 721
in Asian patients, 54
chronic, 55
with isoniazid, in Vietnam, 10
Cyst(s)
bronchogenic
with recurrent pleural effusions, 702
and intralobar sequestration, 2
hydatid, of lung, bronchography after internal suture of pericyst, 20
of lung, 4
pericardial celomic, 702
Cytology in diagnosis of carcinoma, pulmonary, 4
Czechoslovakia
Nitra region, organization and results of antituberculosis vaccination from 1950 to 1956 in, 564
Prague region, mass roentgenography in, 715
Prague and Pilsen regions, vaccination in primary tuberculosis in children and adolescents in, 564
Slovakia
tuberculosis
childhood, mortality of in, 717
mortality and dispensary registers in, 718
tuberculosis in
epidemiologic development of, 718
microbiologic diagnosis of, 706
planning in, 718
Davila-Glover purse-string suture in mitral insufficiency, 561
Decortication for pleural complications of pulmonary tuberculosis, 561
Denmark
cancer of lung in, 45
ornithosis in, 23
Dermatitis, atopic, lung volumes in children with, 58
Diabetes
with fibrocystic disease of pancreas and unusual pulmonary disease, 704
mellitus and tuberculosis, pulmonary, in Sweden, 23
2,4 Diaminoazobenzol-monorhodanid, investigations on, 420
Diaphragm
hernia of
from blunt trauma, 409
congenital and elevation of, 710
neurilemmoma of, causing severe hypertrophic pulmonary osteoarthropathy, 39
rupture of, 710
traumatic, incarceration of stomach and intestine after, 710
Diethylcarbamazine in eosinophilic lung, 555
Dihydrostreptomycin, side effects in sanatorium patients, 56
"Dipasic." *See also* P-aminosalicylic salt of isoniazid
in tuberculosis, pulmonary, chronic, 54
Diverticulosis coli with cholelithiasis and hiatal hernia, 702
Diverticulum, esophageal, esophagobronchial fistula complicating, 711
Drugs, self-administration of, problems in tuberculosis, pulmonary, 720
Dubos medium. *See* Medium(a)
Duct(s), esophagobronchial, congenital, surgical closure of, 17
Dust(s)
cereal grain, causing respiratory allergy in South Africa, 714
coal, 2% quartz in, tissue reaction in rat lungs after inhalation of, 31
Fiberglas®-plastic, biologic action of, 32

Echinococcosis. *See* Mycoses
Edema, pulmonary, roentgenographic diagnosis of, 417
Effusion(s)
hemorrhagic, from extrapleural pneumothorax, prednisolone in, 703
in pericarditis, and Ewart's sign, 403
pleural
in childhood, and its sequels, 557
etiologic distribution of, 554
hemorrhagic, and scurvy, 35
lipids in differential diagnosis of, 576

radioactive phosphorus in diagnosis of, 703
recurrent, bronchogenic cyst with, 702
serous, electrophoretic analysis of, 576
tuberculous, in the aged, 557
Electrocardiography
in embolism, pulmonary, 35
in pectus excavatum, 38
in transitional pulmonary circulation of newborns, 724
Electrophoresis
in cerebrospinal fluid, in meningitis, tuberculous, 44
in effusions, pleural, serous, 576
free micro-, in isolation of tubercle bacilli, 60
in serum changes in silicosis, 38
Embolectomy, pulmonary, 49
Embolism
pulmonary
electrocardiogram in, 35
familial, 703
and infarction, 4, 703
Emphysema
and bronchitis, chronic, 700
bullous, 4
hypertrophic, congenital, 554
localized from tracheal bronchus, 555
and lungs of the aged, 554
lung elasticity in, relaxation pressure curve in, 725
microroentgenography in, 417
obstructive, diffuse, chronic, nonuniformity of pulmonary ventilation in, 727
pulmonary
chronic, epinephrine (racemic) inhalation, in, effects on cardiopulmonary function of normal subjects and patients with, 421
diffuse, and papilledema, 401
localized, in infancy, 4
obstructive, in senile lung, and barrel chest, 36
pathogenesis of, 35
venous admixture in, 723
ulceration, peptic, acute, in, 401
Empyema
pleural, chronic, surgery in, 45
tuberculous, nitrogen mustard in, 571
Encephalomeningitis, tuberculous, in adults, corticotropin in, 43
Encephalopathy in pulmonary disease, 401
Endarteritis obliterans, assessment of, in pulmonary arteries, 575
Endocarditis, bacterial, moniliasis complicating, nystatin in, 53
Endothelioma. *See* Tumor(s)
Energy costs of activities in health and disease, 415
England
Northumberland, pneumoconiosis of coal miners in collieries of, 6
London, East, school in, tuberculous infection in, 51
Eosinophilia
pulmonary infiltration with, due to smoke inhalation, 555
tropical, diethylcarbamazine in, 555
Epinephrine
racemic, inhalation effects on cardiopulmonary function in normal and chronic pulmonary emphysematous subjects, 421
trifluroethane sensitization of dog heart to action of, 711
Epithelium, respiratory tract, smoking, occupation, and residence as factors in squamous metaplasia of, 562
Erythrocytes, changes in, in chronic pulmonary insufficiency, 421
Esophagogastrostomy, pyloroplasty in, with subtotal proximal gastrectomy, 19
Esophagus
achalasia of, surgery in, 708
atresia of, and distant membranous obstruction, 4
and bronchus, fistulas of, 402
carcinoma of, cobalt-60 beam therapy in, 417
carcinoma of, simplified cytodiagnosis in, 711
moniliasis of, roentgenography in, 567
rupture of, spontaneous, 5
Ether analgesia, inspired concentrations, flammability, and levels in arterial blood, 708
Ewart's sign and pericarditis with effusion, 403

Farmer's lung, 36
Fiberglas®-plastic dust. *See* Dust
Fibrosis
cystic
of pancreas
and diabetes, with unusual pulmonary disease, 704
and rectal prolapse, 36
pulmonary, and infiltration of unknown etiology, 558
Fibrothorax and functional retractive syndromes, 560
Finland, tuberculosis mortality and morbidity in, 413
Fistula(s)
arteriovenous, in aneurysm, congenital, of coronary artery, 719
bronchial, secondary to tuberculous spondylitis of thoracic vertebra, 43
esophagobronchial, 402
complicating esophageal diverticulum, 711
nonmalignant, acquired, surgical success in, 46

pulmonary, arteriovenous, and liver abscess, 5
tracheoesophageal
congenital, 711
in adult, 36
tuberculous, trypsin in, 46
Flow pump oxygenator, low metabolism reduced by, and hypothermia, 724
Fluothane®. *See* Trifluroethane
"Flute drainage," 410
Foci, calcified primary, and tuberculous lesions, secondary, pulmonary, 407
France,
Montpellier, Twelfth National Congress of tuberculosis in, 729
Ftivazid®. *See* 3-Methoxy-4-hydroxy-benziliden nicotinic acid hydrazide
Fungus(i)
of Candida group, isolation of, from tuberculous patients, 407
disease from, in northern California, 715
infections
endobronchial, 5
fatal, in sarcoidosis, 7
Furantin®. *See* Nitrofurantoin

Gastrectomy, proximal, subtotal, with esophagogastrostomy, pyloroplasty in, 19
Gastroesophageal sphincter mechanism, 50
Geroderma and nanosomia due to anovaria, 17
Gland(s)
parotid, tuberculosis, nodular, of, 408
pituitary, in sarcoidosis, 404
Goiter, mediastinal, posterior, 402
Gonads, radiation doses to, from diagnostic roentgenographic procedures, 418
Granuloma. *See* Tumor(s)

Hamartoma. *See* Tumor(s)
Health education and tuberculosis, 411
Heart
disease of
acquired, lung changes in, 402
congenital
intracardiac pressure measurement during surgery in, 29
and tuberculosis, pulmonary, 13
of dog, trifluroethane sensitization of to action of epinephrine, 711
and major vessels, disease of, thoracic roentgenographic manifestations in, 417
nonpenetrating traumatic injury of, 411
reaction to selective angiocardiography, 566
"Hematophor" in collection of menstrual blood, 15
Hemolysis, acute, following PAS, 53
Hemosiderosis, pulmonary, idiopathic, iron sequestration in lungs in, 724
Hemorrhage
complications, serious, from isoniazid, 570
during cycloserine treatment, 570
Hernia
diaphragmatic
from blunt trauma, 409
congenital, and elevation of diaphragm, 710
strangulated, 46
hiatal, with cholelithiasis and diverticulosis coli, 702
mediastinal, stratigraphic investigation of, 560
Hilus, measurements of, in diagnosis of primary lung cancer, 567
Histamine in asthma, bronchial, urinary excretion of, 552
Histoplasmosis. *See* Mycoses
Hodgkin's disease, prognosis in, 402
Hormones
adrenocortical, with antimicrobials, in pleurisy, tuberculous, exudative, 53
corticosteroids
aggravating tuberculosis, pulmonary, 9
in meningitis, tuberculous, 14
and corticotropin in tuberculosis, 406
corticotropin
and antimicrobials in tuberculous peritonitis, 568
adrenal secretion in tuberculous patients on, 570
and corticosteroids in tuberculosis, 406
and cortisone in sarcoidosis, pulmonary function before and after, 557
in encephalomeningitis, tuberculous, in adults, 43
in rapid desensitization to PAS, 420
cortisone
with chemotherapy in pleurisy, tuberculous, 53
in fatal fungal infection in sarcoidosis, 7
in histoplasmosis, pulmonary, acute, disseminated, and MRD-112, 5
in tuberculosis
miliary, acute, 407
pulmonary, advanced, major surgery for, 713
hydrocortisone
in meningitis, tuberculous, 44
in pleurisy
exudative, 38
tuberculous, 9
with antimicrobials, in silicotuberculosis, 11
prednisolone
in hemorrhagic effusion caused by extrapleural pneumothorax, 703
prednisone in tuberculous lesion deterioration during chemotherapy, 722
steroids
excretion of, in childhood tuberculosis, 405
in pulmonary disease, 56
Humidification, intarrespiratory, in tracheotomy, simplified method for, 18
Hyaline membrane disease, oxygen treatment in, 729

Hydrocarbon pneumonitis, 556
Hydrocortisone. *See* Hormones
Hydroxydione, cardiovascular, respiratory, and general pharmacologic properties of, 58
2-Hydroxystilbamidine in blastomycosis, toxicity of, 26
Hydrothorax, hepatic, 37
Hyperplasia, lymphatic, and tonsillitis, chronic, 405
Hypertension
  malignant, fatal pulmonary changes during treatment of, 6, 704
  pulmonary experimental production of, 61
  so-called primary pulmonary, 705
Hypertrophy, muscular, assessment of, in pulmonary arteries, 575
Hypoplasia, congenital, 37
Hypothermia
  induced, wound healing after, 18
  metabolism reduced by, and low flow pump oxygenator, 724
  physiologic aspects of, in dogs, 573

Ileum, infarction of, after resection of coarctation of aorta, 19
Infarction and embolism, pulmonary, 4
Influenza
  Asian
    community-wide epidemic of, 37
    pandemic of 1957, 414
      spontaneous pneumothorax in, 7
    S. aureus complicating, 400
    spontaneous pneumothorax in, 705
  epidemic of 1957–1958, mortality in, 414
  lung abscess and pneumonia in, 707
  pneumonia after, familial disposition to, 7
  viruses, 1957, antigenic peculiarities and serologic indexes of immunity, 60
Inhalers, evaluation of, for trichloroethylene, chloroform, and fluothane, 48
Inspiratory positive pressure breathing, circulatory changes associated with, 28
Intermittent positive pressure breathing
  in infants with poliomyelitis, 59
  in postoperative pulmonary complications, 18
Intestine and stomach, incarceration of, after traumatic rupture of diaphragm, 710
Intracranial calcifications after tuberculous meningitis in children, 567
Intralobar sequestration
  and bronchogenic cysts, 2
  bronchopulmonary, 555
  of lung, 8
Intrathoracic disease, scalene node biopsy in, 728
Iron in lungs, sequestration of, in idiopathic pulmonary hemosiderosis, 724
Isoniazid
  active in blood serum, 27
  alone in tuberculosis, pulmonary, in Java, 50
  in BCG-induced immunity in guinea pigs, 575
  damage to eighth cerebral nerve, 28
  influence
    on cortical function, 571
    on pyruvic acid excretion, 571
  levels in children, 721
  in *M. tuberculosis*, effect in, by slide culture method, 27
  PAS added to, effect on blood serum, 26
  prolonged, in tuberculosis, pulmonary, with streptomycin and sodium PAS, 54
  prophylaxis, 52
  resistance in tuberculosis, pulmonary, 571
  -resistant
    BCG, isolation and maintenance of, 727
    strains of *M. tuberculosis* var. *hominis*, pyruvate-containing egg medium in culture of, 574
    tubercle bacilli
      alpha-ethyl-thioisonicotinamide in, 27
      attenuation of pathogenicity in, 729
      highly pathogenic for guinea pigs, 31
      human, virulence studied by whole body homogenization of mice, 60
      in tuberculous meningitis, 559
      in tuberculous meningitis in children, 26
  serious hemorrhagic complications of, 570
  -streptomycin effect on kanamycin-resistant tubercle bacilli, 28
  and streptomycin-resistant tubercle bacilli, distribution of, in lung, 424
  toxicity, pyridoxine in prevention of, 721
  in tuberculosis, pulmonary
    with D-cycloserine, in Vietnam, 10
    early ambulant cases of, 11
    simultaneous with streptomycin and PAS, 572
  in urine, detection of, 56
Isonicotinic acid hydrazide. *See* Isoniazid
Isonicotinylhydrazide, metabolism of, 722

Japanese Association for Tuberculosis, annual report, 13
Java, tuberculosis, pulmonary, in, isoniazid alone in, 50

Kanamycin
  -resistant tubercle bacilli, streptomycin and isoniazid, effect on, 28
  in tuberculosis, pulmonary, 54
17-Ketosteroids
  excretion of, in tuberculosis, and weather, 707
  in tuberculosis, pulmonary, 406
Kidney
  dysplasia of, and tuberculosis, renal, 408
  PAS effects on, 722
Kirchner's medium. *See* Medium(a)
Klebsiella, penicillin influencing emergence of, in sputum, 420
Kveim reaction in sarcoidosis and tuberculosis, 38

Kymography, roentgen, analytic, of normal cardiac cycle, 418

Leiomyosarcoma. *See* Tumor(s)
Leprosy, pulmonary manifestations in, 556
Lesion(s)
  mediastinal
    72 surgical cases, 38
  pulmonary
    metastatic, surgery in, 19
    monolateral, bronchospirometry in, 58
    tuberculous
      and bone and joint tuberculosis in children, 558
      resected, pathology and bacteriology after chemotherapy, 569
      secondary, and calcified primary foci, 407
    indurated, small, relapse of pulmonary tuberculosis in patients with, 559
    prednisone in deterioration of, during chemotherapy, 722
Leukemia and tuberculosis, 406
Lipids
  in differential diagnosis of pleural effusions, 576
  of *M. tuberculosis* var. *Muris-Wells*, 574
Lipoma. *See* Tumor(s)
Liver
  abscess of, and fistulas, pulmonary, arteriovenous, 5
  in sarcoidosis, 404
Lobe(s)
  "excluded," clinical and functional aspects of, 560
  pulmonary, right upper, arterial distribution to, 57
Löffler's syndrome. *See* Eosinophilia
Löwenstein-Jensen medium. *See* Medium(a)
Lung(s)
  of the aged, emphysema and, 554
  agenesis of, 552
  aneurysm of, arteriovenous, in telangiectasis, hereditary, 34
  artery of, abnormal origin of coronary arteries from, 552
  aspergillosis of, 552
    primary, of, 34
  Azygos lobe of, 34
  biopsy, practical method for, 553
  bronchographic agents causing damage in, 719
  cancer of
    in asbestos miners, 23
    cobalt-60 intensive teletherapy in, 417
    diagnosis of, 702
    in females, 714
    primary
      diagnosis of, transverse hilar measurements in, 567
      and tuberculosis, pulmonary, 558
    unusual roentgenographic and clinical findings in, 553
  capacity, total, abdominal surgery, effect on, 58
  capillary blood volume and membrane diffusing capacity of, 57
  carcinoma of
    metastatic, cor pulmonale, subacute, from, 553
    nonresectable, angiocardiography in, 25
    "radical" pneumonectomy in, 409
    and tuberculosis, pulmonary, 706
  cavities, tuberculous, seen roentgenographically and on resected specimens, 24
  circulation of, transitional, in newborns, auscultation and electrocardiography in, 724
  cyst of, 4
    hydatid, bronchography after internal suture of pericyst, 20
    and surgical closure of congenital esophagobronchial duct, 17
  diffusing capacity of,
    for carbon monoxide
      methods of measuring, 57
      measurement by fractional analysis of alveolar air, 30
  diseases of
    from atypical acid-fast bacilli, 414
    chronic, asterixis in, 552
    encephalopathy in, 401
    with fibrocystic disease of pancreas and diabetes, 704
    steroids in, 56
  edema of, roentgenographic diagnosis of, 417
  elasticity of
    in emphysema, relaxation pressure curve in, 725
    in normal and silicotic persons, relaxation pressure curve in, 725
  embolism of
    electrocardiogram in, 35
    familial, 703
    and infarction, 703
  emphysema of
    chronic, epinephrine (racemic) inhalation, in, effects on cardiopulmonary function in normal subjects and patients with, 421
    diffuse, and pappilledema, 401
    obstructive, in senile lung, and barrel chest, 36
    pathogenesis of, 35
  eosinophilic, diethylcarbamazine in, 555
  "Farmer's", 36
  fatal changes of, during treatment for malignant hypertension, 704
  fetal and neonatal, structural appearance of, and capillary erection, 723

function
in sarcoidosis, before and after corticotropin and cortisone, 557
in obese persons, 58
functional shunts of, 29
hamartoma of, 5
in heart disease, acquired changes of, 402
hypertension
experimental production of, 61
primary, of, so-called, 705
infiltration
with eosinophilia due to smoke inhalation, 555
and fibrosis of unknown etiology, 558
rapidly progressive, with fever and coma, 6
insufficiency, chronic, secondary to silo-filler's disease, 6
intralobar sequestration of, 8
involvement of, in panniculitis, nonsuppurative, nodular, relapsing, 705
iron in, sequestration of, in idiopathic pulmonary hemosiderosis, 724
in leprosy, manifestations of, 556
lesions
metastatic, surgery in, 19
monolateral, bronchospirometry in, 58
tuberculous
and bone and joint tuberculosis in children, 558
resected, pathology and bacteriology after chemotherapy, 569
secondary, of, and calcified primary foci, 407
lobe, right upper, arterial distribution to, 57
lymphatic drainage of, in the dog, 576
mast cells of, in experimental silicosis in rat, 62
moniliasis of, 6
mycetoma of, 556
osteoarthropathy, hypertrophic, severe, of, caused by neurilemmoma of diaphragm, 39
peripheral anatomy of, bronchography of, 719
postoperative complications, intermittent positive pressure breathing in, 18
rabbit, experimental formation of tuberculous cavities in, by air-borne infection, 425
rat
tissue reactions produced by calcium fluoride in, 31
tissue reaction in, from 2% quartz in coal dust, 31
resection
anesthesia in, 20
death factors in, 19
extensive, cor pulmonale in cardiopulmonary reserve reduction after, 709
roentgenographic examination during resection, 417
in scleroderma, 7
senile, with barrel chest and obstructive pulmonary emphysema, 36
specimens of, preservation of, by methyl methacrylate, 576
streptomycin- and isoniazid-resistant tubercle bacilli distribution in, 424
torulosis of, 557
transplantation of, in dogs, 29
tuberculosis of
bronchography and anatomy for bronchiectasis in, 410
and cancer of lung, primary, 558
relapse in, 558, 559
surgery of, 410
tumor of
epithelial, malignant, 729
postinflammatory, 8
primary, and tuberculosis, 707
tumor-forming, amyloidosis of, 2
-vascularization, anastomosis of vena cava-pulmonary artery for, 15
veins of
admixture of, in emphysema, silicosis, and silicotuberculosis, 723
drainage of, anomalous, 723
ventilation of
in infants, 726
nonuniformity of, in emphysema, obstructive, diffuse, chronic, 727
in physiologic sleep in normal subjects, 59
ventilatory function, simple instrument for evaluating, 59
vital capacity of, body surface and height as reference medium for, 573
volumes
in dermatitis, atopic, in children, 58
effect of bronchodilator drugs on, in symptom-free asthmatic children, 572
relationships in symptom-free asthmatic children, 422
tests, airway resistance, and -conductance, relationship as to age and body size, 722
of welders, electric-arc and oxyacetylene, 404
Lupus erythematosus, systemic, pleuritis in, 37
Lymphadenitis
purulent, after BCG, 21
suppurative, with caseous tubercle formation after BCG vaccination, 44
trypsin in, 46
Lymphatic drainage, pulmonary, in the dog, 576
Lymph node(s)
biopsy, prescalene, 408
mediastinal, puncture through tracheal carina, 49
M. johnei isolated from cattle, 61
perforation of, tuberculous, into bronchial tree of adult, bronchoscopy in, 405

scalene, nonpalpable, biopsy in, diagnostic value in chest diseases, 49

Measles, tuberculin sensitivity during, 413
Mediastinum
abnormal shadows of, from tortuous thoracic aorta, 416
goiter of, posterior, 402
granulomas of, 5
hernias of, stratigraphic investigation of, 560
masses of, 38
roentgenography of, in silicotuberculosis, pneumomediastinum in, 568
tumor of, azygos vein dilatation simulating, 416
Medium(a)
Dubos
BCG vaccine grown in, first experiences of vaccination with, 564
fluid with Tween®, mycobacteria grown in, determination of viable bacteria in, 423
egg, pyruvate-containing, in culture of isoniazid-resistant strains of *M. tuberculosis* var. *hominis*, 574
Kirchner's, *M. tuberculosis* in, routine isolation of, 423
Löwenstein-Jensen, cycloserine in, resistance determination of tubercle bacilli in, 420
solid and liquid, tests for streptomycin susceptibility of *M. tuberculosis*, 423
Meig's syndrome, 711
Meleinic acid
-hydrazid, 419
-phenylhydrazid, 419
Membrane(s)
mucous, in sarcoidosis, 404
pulmonary, diffusing capacity, measurement of, 57
Meningitis
tuberculous
in children, intracranial calcifications after, 567
corticosteroids in, 14
electrophoretic studies of cerebrospinal fluid in, 44
hydrocortisone in, 44
isoniazid-resistant tubercle bacilli in children with, 26
from isoniazid- and streptomycin-resistant bacilli, 559
present treatment of, 559
working after, 562
Menstrual blood, diagnosis with "hematophor," 15
Mental patients, tuberculosis, pulmonary, in, surgery in, 20
Mercury, metallic, aspiration of, roentgenography in, 556
Mesothelioma. *See* Tumor(s)
Metabolism reduced by hypothermia and low flow pump oxygenator, 724
Metaplasia, squamous, of respiratory tract epithelium, smoking, occupation, and residence as factors in, 562
Metastases
pulmonary
apparent solitary, treatment of, 46
surgery in, 46
3-Methoxy-4-hydroxy-benziliden nicotinic acid hydrazide, estimation of, 571
Methyl methacrylate in lung specimen preservation, 576
Migration as influence upon tuberculosis in cities, 413
Mitral insufficiency, Davila-Glover purse-string suture in, 561
Monge's disease. *See* "Chronic mountain sickness"
Moniliasis. *See* Mycoses
Mortality rate
of tuberculosis
and morbidity, in Finland and Helsinki, 413
increase of, in elderly men, 413
MRD-112. *See* Beta-diethylaminoethylfencholate
Mycetoma. *See* Tumor(s)
Mycobacteria
acid-fast, chromogenic, scrofula from, 707
bovine, tuberculosis from, 717
catalase activity of, 574
dispersing effect of ultrasonics on, 424
grown in Dubos fluid medium with Tween®, determination of viable bacteria in, 423
and mammalian cells in tissue culture, 423
stability of, exposed to ultrasonics, 424
urease activity in, 574
in vivo breakdown, tubercle bacilli, lipid component in, 61
Mycobacterium
johnei
isolation from retropharyngeal and ileocecal lymph nodes and spleen of cattle, 61
mycobactin-containing liquid medium for study of, 61
tuberculosis
var. *hominis*, pyruvate-containing egg medium in culture of isoniazid-resistant strains of, 574
isoniazid effect in, by slide culture method, 27
Kirchner's medium in routine isolation of, 423
var. *Muris-Wells*, lipids of, 574
niacin production by, 60
streptomycin susceptibility of, solid and liquid medium tests for, 423
Mycobactin-containing liquid medium for study of *M. johnei*, 61

Mycoses
aspergillosis
primary, of lung, 34
pulmonary, 552
blastomycosis
2-hydroxystilbamidine in, toxicity of, 26
skin test sensitivity in epidemic area, 50
cryptococcosis
pulmonary (torulosis), resection in, 18
report of Clinical Staff Conference at National Institutes of Health, 553
deep, treatment of, 421
echinococcosis, pulmonary, atypical roentgenograms in, 24
histoplasmosis
bats associated with, 50
human, disease spectrum of, 704
pulmonary, disseminated, acute, cortisone and MRD-112 in, 5
the Walworth, Wisconsin epidemic of, 415
moniliasis
complicating bacterial endocarditis; nystatin in, 53
esophageal, roentgenography in, 567
pulmonary, 6
sarcoidosis, 557
carcinoma, bronchial, co-existing with, 35
cerebral, roentgenographic changes in, 568
fatal fungal infection in, 7
lung function in before and after corticotropin and cortisone, 557
pine pollen in, 562
and pituitary gland involvement, 404
pulmonary, photofluorography in, 563
splenic, hepatic, mucous-membrane, retinal, and joint manifestations in, 404
systemic, and tuberculosis, 15
and tuberculosis, Kveim reaction in, 38
Mycostatin®. *See* Nystatin
Myocarditis, Fiedler's, fatal, following intramuscular streptomycin, 420

Nanosomia and geroderma due to anovaria, 17
National Institutes of Health, Clinical Staff Conference, report on cryptococcosis, 553
Neurilemmoma. *See* Tumor(s)
Nerve(s)
eighth cerebral, isoniazid damage to, 28
phrenic, tumor, neurogenic, of, 39
Neuritis. *See* Neuropathy
Neurofibromatosis, clinical and roentgenographic manifestations of, 403
Neuropathy, peripheral, with bronchiectasis, 401
Niacin, *M. tuberculosis* production of, 60
Nitrofurantoin as a coccidioidal fungicide, 53
Nitrofurazone in tumor, testicular, metastatic, 56
Nitrogen mustard in tuberculous empyema, 571
Node(s), scalene, biopsy in intrathoracic disease, 728
Noscapine in respiratory response to carbon dioxide, 59
Nucleus(i), intra-, inclusion body, in fatal infantile pneumonia from adenovirus, 403
Nystatin, crystalline, in moniliasis complicating bacterial endocarditis, 53

Obesity, pulmonary function in persons with, 58
Oleothorax postpneumonectomy, 48
Ornithosis in Denmark, 23
Osteoarthropathy
pulmonary, hypertrophic
with carcinoma, bronchial, vagotomy in, 702
severe, caused by neurilemmoma of diaphragm, 39
Oxygen in hyaline membrane disease, 729
Ozone toxicity in young chicks, 32

P^{32} in tubercle bacilli virulence studies, 61
Pancreas
cystic fibrosis of
and diabetes, with unusual pulmonary disease, 704
and rectal prolapse, 36
Pancreatin in tubercle bacilli cultivation, with cetyltrimethylammonium bromide, 12
Pancytopenia and thymoma, 39
Panniculitis, nonsuppurative, nodular, relapsing, with lung involvement, 705
Pappilledema and emphysema, pulmonary, diffuse, 401
Para-aminosalicylic acid
adjunct to isoniazid, effect on blood serum, 26
corticotropin in rapid desensitization to, 420
excretion of, and its acetyl derivative, 572
hemolysis, acute, following, 53
renal failure from, 722
sodium, prolonged, in tuberculosis, pulmonary, with streptomycin and isoniazid, 54
in tuberculosis, pulmonary, simultaneous with streptomycin and isoniazid, 572
Parallergy in tuberculosis, pulmonary, 405
Particle-sizing method for aerosols and fine powders, 23
Patent ductus arteriosus, "atypical," surgery in, 708
Pectus excavatum, electrocardiographic changes in, 38
Penicillin, influence on emergence of Klebsiella in sputum, 420
Pericarditis with effusion, and Ewart's sign, 403
Pericardium
biopsy of, with Vim-Silverman needle, 408
drainage, self-cleansing, of, 410

Pericyst, internal suture of, bronchography after, 20
Peritonitis, tuberculous, antimicrobials and corticotropin in, 568
Peroxidase activity of tubercle bacilli, 728
Phagocytosis of tubercle bacilli, *in vitro*, with and without antimicrobials, 60
Phosphorus, radioactive, in pleural effusion diagnosis, 703
Photofluorography. *See* Roentgenography
Photography, bronchial, with simple apparatus, 411
Physical therapy in chronic bronchopulmonary disorders, 3
Pine pollen in sarcoidosis, 562
Placental extracts in tuberculosis, pulmonary, streptomycin-resistant, 53
Plasma. *See also* Blood
  adenosine deaminase activity in, in cancer patients, 400
Pleura
  complications of, in tuberculosis, pulmonary, decortication for, 561
  drainage, self-cleansing, of, 410
  endothelioma of, diagnosis of, 703
  parietal, biopsy, needle, of, 408
  rupture of angiomatous malformation of, in newborn, 556
Pleural effusion. *See* Effusion(s)
Pleural fluid, sugar content of, 403
Pleurisy
  with effusion, in childhood, and its sequels, 557
  exudative, hydrocortisone in, 38
  tuberculous
    cortisone and chemotherapy in, 53
    with effusion, in the aged, 557
    exudative, adrenocortical hormones and antimicrobials in, 53
    hydrocortisone, local, in, 9
Pleuritis
  in lupus erythematosus, systemic, 37
  mediastinalis superior in adults, 556
Plombage, extraperiosteal, in tuberculosis, pulmonary, 711
Pneumoconiosis
  of coal miners in Northumbrian collieries, 6
  in grain, oil, and flour workers, 715
Pneumolysis cavities, management of, 409
Pneumomediastinum in roentgenography of mediastinum in silicotuberculosis, 568
Pneumonectomy
  in leiomyosarcoma, 20
  "radical," in carcinoma of lung, 409
Pneumonia
  infantile, fatal, from adenovirus, 403
  and lung abscess in influenza, 707
  pneumocystic, causing death of surgically treated newborn, 705
  pneumocystis, 705
  post-influenzal, familial disposition to, 7
  staphylococcic, in infancy and childhood, 403
Pneumonitis
  chronic, nonspecific, surgery in, 19
  hydrocarbon, 556
  interstitial, granulomatous, acute (Farmer's lung), in agricultural workers, 36
Pneumothorax
  extrapleural
    causing hemorrhagic effusion, prednisolone in, 703
    recent results with, 561
  progressive, acute, respiratory response to, 421
  residual air in, 59
  spontaneous
    and 1957 influenza, 7
    in influenza, Asian, 705
Poliomyelitis, intermittent positive pressure respiration in infants with, 59
PPD. *See* Tuberculin
Prednisolone. *See* Hormones
Pregnancy and tuberculosis, pulmonary, 13
Pressure ventilation, positive-negative, modified Ayre's T-piece in, 727
Promethazine hydrochloride in bronchoscopy, 20
Pubis, tuberculosis of, 408
Pulmonary dynamics, field testing of, 421
Pulmonary insufficiency, chronic, erythrocyte changes in, 421
Pulmonary shunts, 29
Pulmonary venous return, anomalous, total, complete correction of, 48
Purpura and tuberculosis, 13
Pyloroplasty in subtotal proximal gastrectomy with esophagogastrostomy, 19
Pyrazinamide
  antituberculous effect of, 28
  in tuberculosis, pulmonary, 572
Pyridoxine in isoniazid toxicity prevention, 721
Pyruvic acid, excretion of, influence of isoniazid on, 571

Quartz in coal dust, tissue reaction in rat lungs after inhalation of, 31

Radiation
  in diagnostic roentgenographic procedures, 418
    dose measurement techniques in, 418
    gonadal exposure dose from, 418
  therapy in carcinoma of bronchial stump, recurrent, 25
  and tuberculosis chest roentgenographic examinations, 25
Radioactive phosphorus
  -labeled tubercle bacilli, distribution of in guinea pig after subcutaneous infection, 30

Ramibacterium pleuriticum, pathogenic properties of, 574
Rectum, prolapse of, in cystic fibrosis of pancreas, 36
Red cells. *See* Erythrocytes
Relapse of tuberculosis, pulmonary, chronic, in adults, 11
Resection
in bronchiectasis, long-term results of, 709
of coarctation of aorta, infarction of ileum after, 19
in cryptococcosis, pulmonary (torulosis), 18
pulmonary
anesthesia in, 20
death factors in, 19
extensive, cor pulmonale in cardiopulmonary reserve reduction after, 709
roentgenography during, 417
in tuberculosis, 16
sleeve, of main-stem bronchus in bronchial adenoma, 18
in tuberculosis, 712
Residual air in pneumothorax, 59
Respiration
artificial manual and intermittent positive-pressure compared, 725
complications in postoperative period, 49
intermittent positive pressure in poliomyelitis, infants with, 59
physical investigation of, 725
rebreathing, determination of mean venous blood carbon dioxide tension from exponential carbon dioxide rise during, 422
studies in children, symptom-free, asthmatic, lung volumes in, 422
Respiratory
dead space, and alveolar ventilation, 725
failure, emergency treatment and transport of patients with, 725
function and age in normal men at work, 59
paralysis, new method for measurement of partial pressure of blood gases in children with, 572
pathology and altitude, 726
resistance, time-marked respiratory pressure volume loops in, 726
response to acute progressive pneumothorax, 421
studies in children, asthmatic and healthy, maximal breathing capacity in, 726
tract giant-cell granuloma of, 39
Retina in sarcoidosis, 404
Rib(s), anomalies of, found during mass roentgenography, 716
Roentgenography
in agammaglobulinemia, 415
anatomy, studies of, compared with, in tuberculosis mortality, 24
angiocardiography
in Bland-White-Garland syndrome, 566
in carcinoma, pulmonary, nonresectable, 25
as diagnostic procedure, 418
intravenous, analysis of 600 cases, 566
selective, reaction of heart to, 566
angiography
of anomalous artery in intralobar bronchopulmonary sequestration, 719
carotid, with sodium acetrizoate, blood pressure and heart rate responses in, 566
in arteries, subclavian, 416
bronchography
agents used in, damage to lung from, 719
blood iodine concentration after, 566
of peripheral pulmonary anatomy, 719
in cancer of lung, unusual findings with, 553
in cavities, tuberculous, pulmonary, 24
of chest, comparison of simultaneous 10 × 10- and 35 × 35-cm. films, 419
in diagnosis of aneurysm, traumatic, chronic, acute, of thoracic aorta, 416
diagnostic procedures of
gonadal exposure dose from, 418
millisecond timing and angiocardiography, comparison of dynapulse and impulse timing, 418
radiation doses in, 418
radiation dose measurement techniques in, 418
in echinococcosis, pulmonary, 24
in edema, pulmonary, diagnosis of, 417
in heart and great vessel disease, thoracic manifestations, 417
of lung
during resection, 417
of electric-arc and oxyacetylene welders, 404
mass
in the Prague region, 715
rib anomalies found during, 716
of mediastinum in silicotuberculosis, pneumomediastinum in, 568
in metallic mercury aspiration, 556
micro-, in emphysema, 417
in moniliasis, esophageal, 567
in neurofibromatosis, 403
photofluorography
observer error in multiple interpretation in, 567
in sarcoidosis, pulmonary, 563
and radiation in tuberculosis chest examinations, 25
of right cardiophrenic angle, shadows in, caused by intrapleural lipoma, 418
in sarcoidosis, cerebral, 568
in silicotuberculosis, 568
venography, internal mammary, 24

Sarcoidosis. *See* Mycoses
Scleroderma, the lung in, 7
Scotland, Dumfriesshire, respiratory symptoms, bronchitis, and disability in agricultural community in, 714
Scrofula from chromogenic acid-fast mycobacteria, 707
Scurvy and effusion, pleural, hemorrhagic, 35
Septum, atrial and ventricular defects of, open intracardiac repair of, with extracorporeal circulation, 712
Seroprotein and seroglycoprotein pattern changes in pulmonary tuberculosis after surgical and medical treatment, 559
Serum changes in silicosis, electrophoresis in, 38
Silicosis
-complicated tuberculosis, pulmonary, long-term chemotherapy of, 419
a continuing problem, 415
experimental in rat, pulmonary mast cells in, 62
fire clay, experimental, 62
in fireproof ceramic industry, 23
lung elasticity in, relaxation pressure curve in, 725
pulmonary venous admixture in, 723
rapidly evolving, electrophoresis in serum changes in, 38
in tuberculosis, pulmonary, 707
Silicotuberculosis
pneumomediastinum in roentgenography of mediastinum in, 568
prednisolone and antimicrobials in, 11
pulmonary venous admixture in, 723
roentgenography in, 568
Silo-filler's disease, pulmonary insufficiency, chronic, secondary to, 6
Sinus(es), facial, secondary to tuberculosis pulmonary, 707
Sleep, physiologic, alveolar gas tensions, pulmonary ventilation, and blood pH during, 59
Slide culture method. *See* Tests
Smoke inhalation, pulmonary infiltration with eosinophilia due to, 555
Smoking
and cancer and coronary artery disease, 715
tobacco in squamous metaplasia of respiratory tract epithelium, 562
Sodium acetrizoate, carotid angiography with blood pressure and heart rate responses in, 566
South Africa, respiratory allergy from cereal grain dust in, 714
Spleen
M. johnei isolated from cattle, 61
in sarcoidosis, 404
Spondylitis
tuberculous
differential diagnosis in, 14
of thoracic vertebra, bronchial fistula secondary to, 43
Staphylococcus aureus
complicating Asiatic influenza, 400
in pneumonia in infancy and childhood, 403
Stenosis
bronchial, kinked bronchus simulating, 17
subaortic, 38
Steroids. *See* Hormones
Stomach and intestine, incarceration of, after traumatic rupture of diaphragm, 710
Streptoduocin, auditory changes from, 52
Streptomycin
auditory changes from, 52
intramuscular, causing fatal Fiedler's myocarditis, 420
-isoniazid effect on kanamycin-resistant tubercle bacilli, 28
and isoniazid-resistant tubercle bacilli, distribution of, in lung, 424
M. tuberculosis susceptibility to, tests on solid and liquid medium for, 423
prolonged, in tuberculosis, pulmonary, with sodium PAS and isoniazid, 54
-resistant
bacilli, tuberculous meningitis from, 559
tuberculosis, pulmonary placental extracts in, 53
tuberculin detoxication by incubation in presence of, 574
in tuberculosis, pulmonary, simultaneous with PAS and isoniazid, 572
Streptovaricin, antituberculous activity of, in guinea pigs, 28
Sugar content of pleural fluid, 403
Surgery
abdominal, effect on lung capacity, total, 58
in achalasia of esophagus, 708
in atrial and ventricular septal defects with extracorporeal circulation, 712
in bronchiectasis, 44
in empyema, pleural, chronic, 45
in fistula, esophagobronchial, nonmalignant, acquired, 46
major, in tuberculosis, pulmonary, advanced, cortisone in, 713
in metastases, pulmonary, 46
in patent ductus arteriosus, "atypical," 708
in pneumonitis, chronic, nonspecific, 19
resectional, in bronchiectasis, in tuberculosis, pulmonary, 410
thoracic
anesthesia in, 20
postoperative infections in, 15
in tuberculosis, pulmonary, 47
in tuberculosis
primary, in children, 561
pulmonary, 410

and other chest conditions, analysis of, 47
in mentally ill, 20
silicotic, 712
Sweden, diabetes mellitus and tuberculosis, pulmonary, in, 23

Tebanyl-Leo.® *See* *p*-Aminosalol
Telangiectasis, hereditary, aneurysm, pulmonary, arteriovenous in, 34
Test(s)
airway resistance, -conductance, and lung volume relationship as to age and body size, 722
of alveolar ventilation and physiologic dead space, 726
BCG in practice, 713
cadmium in tuberculosis, pulmonary, 12
maximal breathing capacity in asthmatic children, 726
multiple-puncture depot tuberculin (PPD) cream, in man, 716
positive-negative pressure ventilation, modified Ayre's T-piece in, 727
provocation, in asthma, bronchial, 57
relaxation pressure curve
in lung elasticity
in emphysema, 725
of normal and silicotic persons, 725
skin, blastomycin, follow-up of, in epidemic area, 50
slide culture method
demonstration of immunity to tuberculosis by, 575
in effect of isoniazid on *M. tuberculosis*, 27
for tuberculosis, pulmonary, 43
Tetracycline in bronchitis, chronic, 722
Thioamid of alpha-ethyl-isonicotinamide in tubercle bacilli, isoniazid-resistant, 27
Thiopneumoconiosis, 23
Thoracoplasty, extrapleural, in tuberculosis, pulmonary, 17
Thyoma. *See* Tumor(s)
Tissue(s)
carbon dioxide content of, serial changes in, during acute respiratory acidosis, 57
culture, mycobacteria and mammalian cell relationship in, 423
Tonsillitis, chronic, and lymphatic hyperplasia, 405
Torulosis. *See also* Mycoses (Cryptococcosis)
pulmonary, 557
Trachea, plasma cell tumor of, 705
Tracheotomy, intrarespiratory humidification in, 18
Trauma, blunt, diaphragmatic hernia from, 409
Trichloroethylene inhalers evaluated for, 48
Trifluorethane
inhalers evaluated for, 48
-sensitization of dog heart to action of epinephrine, 711
Triurol®. *See* Sodium acetrizoate
Trochanter, greater, tuberculosis of, 408
Trypsin
intramuscular, in bronchitis, chronic, 701
in tuberculosis, lymphadenitis, and tuberculous fistulas, 46
Tubercle, caseous formation of, in lymphadenitis, suppurative, after BCG, 44
Tubercle bacillus(i)
control of contamination in cultivation of, 574
effect of cancer cells, human, in tissue culture on, 422
electrophoresis, free micro-, in isolation of, 60
human, isoniazid-resistant strains, virulence studied by whole-body homogenization of mice, 60
intrapulmonary injection of, tissue reaction caused by, 425
isoniazid-resistant
alpha-ethyl-thioisonicotinamide in, 27
attenuation of pathogenicity in, 729
highly pathogenic for guinea pigs, 31
and streptomycin-resistant tuberculous meningitis from, 559
in tuberculous meningitis in children, 26
kanamycin - resistant, streptomycin - isoniazid effect on, 28
P^{32}-labeled, after subcutaneous infection, distribution in guinea pig, 30
pancreatin and cetyltrimethylammonium bromide in cultivation of, 12
peroxidase activity of, 728
phagocytosis, *in vitro*, with and without antimicrobials, 60
resistance determination of, in cycloserine added to Löwenstein-Jensen medium, 420
streptomycin- and isoniazid-resistant, distribution of, in lung, 424
virulence of, P^{32} used as tracer in studies of, 61
virulent
tuberculin allergy from, 413
tuberculin allergy in guinea pigs after BCG vaccination and subsequent infection with, 575
Tuberculin
allergy
from BCG and virulent tubercle bacilli, 413
in guinea pigs, after BCG vaccination and subsequent infection with virulent tubercle bacilli, 575
detoxication by incubation in presence of streptomycin, 574
-negative tuberculosis, 8
Old, in tuberculosis, portio, cure of, 15

PPD
age factors in reaction to, 716
multiple-puncture depot, cream tests in man, 716
skin reactions from, 562
sensitivity
during measles, 413
epidemiologic studies of, 716
trace elements in preparation of, 728
Tuberculosis
abeyant, 563
adenopathies, mediastinal, in, etiologic diagnosis of, 42
and aged, 412
in Australia, 21
in BCG-vaccines, 21
bone and joint, 14
in BCG-vaccinated children, 713
functional results in children, 563
and tuberculous pulmonary lesions in children, 558
from bovine mycobacteria, 717
Candida fungi in patients with, isolation of, 407
chemotherapy
fatal complications of, 569
in infants, 55
in childhood, 40
diagnosis, treatment, and prophylaxis problems, 717
mortality in Slovakia, 717
recurrences after chemotherapy in, 25
and steroid excretion, 405
in cities, migration as influence on, 413
congenital, 44
corticotropin and corticosteroids in, 406
cycloserine in, neuropsychiatric accidents from, 26
in Czechoslovakia
epidemiologic development of, 718
microbiologic diagnosis of, 706
experimental, chemotherapy, prophylactic, in, 570
health education and, 411
immunity to, demonstration of, by slide culture method, 575
and leukemia, 406
in London school children, 51
long-range plan for research and control of, 564
in lymph node perforation into bronchial tree, bronchoscopy in, 405
management of, significance to industry, 21
menstrual blood diagnosis in, 15
miliary, acute, cortisone in, 407
fatal, in immunized child, 44
morbidity and alcoholism, 563
mortality
clinical and social study of, 22
and dispensary-registers in Slovakia, 718
increase in elderly men, 413
and morbidity, in Finland and Helsinki, 413
roentgenographic and anatomic data compared in, 24
nodular, of parotid gland, 408
of pelvic bone, with hip joint involvement, 15
planning in Czechoslovakia, 718
portio, OT-cure of, 15
primary
in children, surgery in, 561
chronic, and evolutive, correlated with bronchial pathology, 41
vaccination in, in children and adolescents in Prague and Pilsen regions, 564
prognosis after treatment, medical survey, 22
of pubis, 408
pulmonary
active, from pulmonary infiltration and fibrosis of unknown etiology, 558
in adults, negligence as major cause of relapse in, 40
advanced, cortisone in major surgery for, 713
bronchial disease and bronchography in, 52
bronchography in, 52
for bronchiectasis in, 410
bronchoscopy in, 12
cadmium reaction in, 12
calcium B-PAS in, drug resistance to, 568
and cancer of lung, primary, 558
Candida in, 706
and carcinoma, bronchogenic, in, 41
pulmonary, 706
chemotherapy
intravenous, in, 569
for outpatients, 718
chest pain in, 406
chronic
in adults, 11
cycloserine in, 55
"dipasic" in, 54
cor pulmonale in, 42
corticosteroids aggravating, 9
cycloserine in, 10, 570, 721
in Asian patients, 54
D-cycloserine in, with isoniazid, in Vietnam, 10
decortication for pleural complications of, 561
and diabetes mellitus in Sweden, 23
drug self-administration problems in, 720
extraperiosteal plombage in, 711
extrapleural thoracoplasty in, 17
far advanced, prolonged antimicrobial therapy in, 9
and heart disease, congenital, 13
isoniazid in early ambulant cases of, 11
isoniazid resistance in, 571
in Java, isoniazid alone in, 50
kanamycin in, 54

17-ketosteroid in, 406
in mentally ill, surgery in, 20
new compound in, 55
p-aminosalol in, 26
parallergy in, 405
and pregnancy, 13
primary, significant, in young contacts, 51
prolonged streptomycin, sodium PAS, and isonizaid in, 54
pyrazinamide in, 572
relapse in, 558, 559
sero- and seroglycoprotein pattern changes after surgical and medical treatment, 559
in silicosis, 707
complicated, long-term chemotherapy of, 419
surgery in, 712
simultaneous intravenous streptomycin, PAS, and isoniazid in, 572
sinuses, facial, secondary to, 707
streptomycin-resistant, placental extracts in, 53
surgery of, 410
analysis of, 47
tests for, 43
thoracic surgery in, 47
and ulcer, peptic, 42
uropepsin excretion in, 43
and purpura, 13
recent trends in South Australia, 565
relapses of, in females, psychologic factors in, 563
renal, and kidney dysplasia, 408
resection in, 712
pulmonary, in, 16
roentgenographic chest examinations and radiation, 25
and sarcoidosis
kveim reaction in, 38
systemic, 15
school epidemics of, 412
surveys, mass, statistical results of, 52
treatment of today, 40
of trochanter, greater, 408
trypsin in, 46
tuberculin-negative, 8
and tumor of lung, primitive, 707
and unsocial elements of the community, 412
Twelfth National Congress of, in Montpellier, 729
vaccination against, first experiences using BCG vaccine grown in Dubos medium, 564
weather and excretion of 17-ketosteroids in, 707
Tumor(s)
adenoma
bronchial
metastasizing, and carcinoid syndrome, 3
sleeve resection of main-stem bronchus in, 18
carcinoid
and adenoma, bronchial, metastasizing, 3
bronchial, clinical findings in, 400
desmoid, of chest, 411
endothelioma, so-called "pleural," diagnosis of, 703
-forming amyloidosis of lung, 2
granuloma
giant-cell (Wegener's granulomatosis) of respiratory tract, 39
of mediastinum, 5
hamartoma of lung, 5
leiomyosarcoma, pneumonectomy in, 20
-like formation frequency in bronchiectasis, 560
lipoma, intrapleural, in right cardiophrenic angle roentgenographic shadows, 418
of lung
epithelial, malignant, 729
postinflammatory, 8
primary, and tuberculosis, 707
of mediastinum, azygos vein dilatation simulating, 416
mesothelioma, pleural, 556
mycetoma of lung, 556
neurilemmoma of diaphragm, causing severe hypertrophic pulmonary osteoarthropathy, 39
neurogenic, of phrenic nerve, 39
plasma cell, of trachea, 705
testicular, metastatic, nitrofurazone in, 56
thymic, histology and behavior of, 47
thymoma
benign, and aregenerative anemia, 34
and pancytopenia, 39
Tween®, Dubos fluid medium with, mycobacteria grown in, determination of viable bacteria in, 423

Ulcer
peptic
acute, in emphysema, 401
and tuberculosis, pulmonary, 42
Ultrasonics
dispersing effect of, on mycobacteria, 424
stability of mycobacteria exposed to, 424
United States
California, northern, fungal disease in, 715
"chronic mountain sickness" in, 401
Wisconsin, Walworth, histoplasmosis, epidemic of, 415
Urease activity of mycobacteria, 574
Urine, isoniazid detection in, 56
Uropepsin excretion in tuberculosis, pulmonary, 43

Vaccination
antituberculosis, organization and results of,

from 1950 to 1956 in Nitra region (Slovakia), 564
BCG, role of, 713
of newborns, with intracutaneous and peroral doses of BCG vaccine, 565
in tuberculosis
first experiences, using BCG vaccine grown in Dubos medium, 564
primary, in children and adolescents in Prague and Pilsen regions, 564
Vagotomy in osteoarthropathy, pulmonary, hypertropic, with bronchial carcinoma, 702
Vein(s)
azygos, dilatation simulating mediastinal tumor, 416
pulmonary
admixture of, in emphysema, silicosis, and silico-tuberculosis, 723
anomalous, drainage of, 723
Vena cava, superior, syndrome, aortic graft in, 16
Venography. *See* Roentgenography
Ventilatory failure, carbon dioxide analyzer in rapid diagnosis of, 30
Vertebra, thoracic, tuberculous spondylitis of, bronchial fistula secondary to, 43
Vessels, major, and heart, disease of, thoracic roentgenographic manifestations in, 417
Vietnam, tuberculosis, pulmonary, in, D-cycloserine and isoniazid in, 10
Vim-Silverman needle in pericardial biopsy, 408
Vital capacity difference, 60

Wales, New South, cough and climate relationship in a smogless coastal district of, 415
Weather in tuberculosis and excretion of 17-ketosteroids, 707
Wegener's granulomatosis. *See also* Tumor(s)
Wegener's granulomatosis, 404
of respiratory tract, 39
Welders, electric-arc and oxyacetylene lungs of, 404